UNIVERSITY OF HELSINKI

DELAYED PUBERTY

ETIOLOGY, OUTCOME AND INTERACTIONS WITH GROWTH

TERO VARIMO

HELSINKI 2016

Pediatric Graduate School
Doctoral Programme in Clinical Research
Children's Hospital
Helsinki University Hospital and
University of Helsinki
Helsinki, Finland

DELAYED PUBERTY

ETIOLOGY, OUTCOME AND INTERACTIONS WITH GROWTH

Tero Varimo

ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Medicine of
the University of Helsinki, for public examination at the Niilo Hallman Auditorium,
Children's Hospital, on January 20th 2017, at 12 noon.

Helsinki 2016

Supervised by

Professor Taneli Raivio
Children's Hospital
Helsinki University Hospital
and Department of Physiology
Faculty of Medicine
University of Helsinki
Helsinki, Finland

MD, PhD Matti Hero
Children's Hospital
Helsinki University Hospital
Helsinki, Finland

Reviewed by

Professor Juha Tapanainen
Department of Obstetrics and
Gynecology
Helsinki University Hospital
University of Helsinki
Helsinki, Finland

Docent Marja Ojaniemi
Oulu University Hospital
and PEDEGO Research Unit, Medical
Research Center Oulu,
University of Oulu
Oulu, Finland

Official opponent

Docent Antti Perheentupa
Department of Obstetrics and Gynecology
Turku University Hospital
University of Turku
Turku, Finland

ISBN 978-951-51-2838-6 (Paperback)
ISBN 978-951-51-2839-3 (PDF)

Painosalama Oy
Helsinki 2016

To Eveliina and Joonatan

TABLE OF CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following articles which are referred to in the text by Roman numerals I-V:

I. **Varimo T**, Dunkel L, Vaaralahti K, Miettinen PJ, Hero M, Raivio T. Circulating makorin ring finger protein 3 (MKRN3) levels decline in boys before the clinical onset of puberty. *Eur J Endocrinol.* 2016;174:785-90.

II. **Varimo T,** Miettinen PJ, Känsäkoski J, Raivio T, Hero M. Congenital hypogonadotropic hypogonadism, functional hypogonadotropism, or constitutional delay of growth and puberty? An analysis of a large patient series from a single tertiary center. *Hum Reprod*. In press.

III. **Varimo T**, Hero M, Laitinen EM, Miettinen PJ, Tommiska J, Känsäkoski J, Juul A, Raivio T. Childhood growth in boys with congenital hypogonadotropic hypogonadism. *Pediatr Res*. 2016;79:705-9.

IV. Hero M, Laitinen EM, **Varimo T**, Vaaralahti K, Tommiska J, Raivio T. Childhood growth of females with Kallmann syndrome and FGFR1 mutations. *Clin Endocrinol (Oxf)*. 2015;82:122-6.

V. **Varimo T**, Hero M, Laitinen EM, Sintonen H, Raivio T. Health-related quality of life in male patients with congenital hypogonadotropic hypogonadism. *Clin Endocrinol (Oxf)*. 2015;83:141-3.

ABBREVIATIONS

AI	aromatase inhibitor
AMH	anti-müllerian hormone
ANOS1	*anosmin 1*
ARC	arcuate nucleus
BDI	beck's depression index
BMD	bone mineral density
BMI	body mass index
CAH	congenital adrenal hyperplasia
CDGP	constitutional delay of growth and puberty
CHD7	*chromodomain helicase DNA-binding protein 7*
CHH	congenital hypogonadotropic hypogonadism
CI	confidence interval
CNS	central nervous system
CPP	central precocious puberty
DP	delayed puberty
DYN	dynorphin
ELISA	enzyme-linked immunosorbent assay
FGFR1	*fibroblast growth factor receptor 1*
FHH	functional hypogonadotropic hypogonadism
FSH	follicle-stimulating hormone
GABA	gamma-amino butyric acid
GH	growth hormone
GnRH	gonadotropin-releasing hormone
GNRHR	*gonadotropin-releasing hormone receptor*
GPR54	*g protein-coupled receptor 54*
hCG	human chorionic gonadotropin
HPG axis	hypothalamic-pituitary-gonadal axis
Hyper H	hypergonadotropic hypogonadism
HRQoL	health-related quality of life
ICD	international classification of diseases
IGF	insulin-like growth factor
IGSF10	*immunoglobulin superfamily member 10*
ISO-BMI	age- and sex-adjusted body mass index
ISS	idiopathic short stature
KISS1R	*kisspeptin 1 receptor*
KNDy	kisspeptin/neurokinin B/dynorphin
KS	Kallmann syndrome
LH	luteinizing hormone
Lz	letrozole
MKRN3	*makorin ring finger protein 3*
MPH	mid-parental target height

MRI	magnetic resonance imagining
NKB	neurokinin B
OR	odds ratio
PHH	permanent hypogonadotropic hypogonadism
PROK2	*prokineticin 2*
ROC	receiver operating characteristic
SD	standard deviation
SDS	standard deviation score
TAC3	*tachykinin 3*
TAC3R	*tachykinin 3 receptor*
TS	Turner syndrome
VNN	vomero-nasal nerve

ABSTRACT

During puberty, adolescents achieve reproductive capability and attain adult height. An intact hypothalamic-pituitary-gonadal (HPG) axis is crucial for the normal onset of puberty. Pubertal timing is influenced by genetic and environmental factors such as nutrition and stress. However, the exact mechanisms that trigger puberty remain elusive. A recent finding of a paternally-inherited loss-of-function mutation in *MKRN3* gene in patients who were diagnosed with central precocious puberty suggests that key genes regulate the central restrain on the HPG axis during childhood, and that the loosening of the restrain precedes the onset of puberty. At the moment, the methods to measure the amount of the central restrain are limited to only observing its influence further down the HPG axis. Thus, a marker which reflects the strength of the central restrain directly could aid in the differential diagnosis and the follow-up of pubertal disorders. A variety of diseases can delay puberty, but it is particularly important to early identify the patients who suffer from acquired or congenital hypogonadotropic hypogonadism (CHH) which results from the absent or impaired functions of gonadotropin-releasing hormone. This congenital sex steroid deficiency predisposes to long-term consequences, but very little is known about its role in the modulation of growth during early infancy and its impact on health-related quality of life (HRQoL) in adulthood.

In the first study, circulating MKRN3 levels were measured in boys with idiopathic short stature (ISS) (n=30) before and after the onset of puberty. Sixteen boys were treated with an aromatase inhibitor letrotzole (Lz) for two years in an attempt to increase their adult height. In boys, serum levels of MKRN3 declined faster before than after the clinical onset of puberty. The boys who were treated with Lz had a slower decrease in MKRN3 levels than placebo-treated boys, and the decline in MKRN3 had a reciprocal relationship with the clinical and the biochemical markers (*i.e.* testis size, luteinizing hormone, inhibin B, testosterone) of puberty onset.

In the second study, the diagnoses that underlie delayed puberty (DP) and its outcome predictors were investigated in a large (n=244) series of patients who were examined for delayed puberty at the Helsinki University Central Hospital between 2004 and 2014. A multitude of different diagnoses were found to cause DP, albeit constitutional delay of growth puberty (CDGP) was the most common cause in both sexes. Further, annual growth velocity, a history of prior cryptorchidism, and a positive family history of DP were useful for the differential diagnosis of DP. Additionally, measuring testicular size with a ruler emerged as an effective parameter which could aid in differentiating the prepubertal boys with CHH from those with CDGP.

The third and fourth study consisted of growth charts of 42 patients (36 boys and 6 girls) with CHH, and evaluated the influence of congenital sex steroid deficiency on growth from birth length to adult height. In girls with CHH and *FGFR1* mutations, the growth rate decreased from mid-childhood. Conversely, the boys with CHH experienced a length deflection during the first six months of life (minipuberty), which was likely to result from deficient testosterone surge during

the same period. In both sexes, the height standard deviation score nadir was reached at the age of pubertal induction.

In the fifth study, the HRQoL was measured in men with CHH (n=30) with the generic 15D questionnaire. The men with CHH experienced an impaired HRQoL, especially in the dimensions of depression and distress. The finding that the age of diagnosis correlated negatively with HRQoL scores supports the view that timely diagnosis of CHH is an important goal when evaluating a patient with late puberty.

In summary, sex steroids modulate growth from early infancy to adulthood. In boys, circulating MKRN3 levels decline before the clinical onset of puberty which supports the current view that *MKRN3* is a key regulator of pubertal onset. Our findings suggest a relationship between estrogen and MKRN3 secretion. Additionally, the results pave the way for MKRN3 as a novel biochemical marker that may turn out to be useful in the evaluation of pubertal disorders in a research setting. Many diseases can delay pubertal maturation, but CDGP is the most common cause in Finland. In men, the timely diagnosis of CHH is crucial to avoid the long-term adverse effects of sex steroid deficiency on HRQoL. Finally, the results not only emphasize the importance of thorough review of medical history and a careful physical examination when evaluating an adolescent with DP, but also bring new tools in clinical decision-making.

FINNISH SUMMARY (TIIVISTELMÄ)

Murrosiässä nuori siirtyy lapsuudesta aikuisuuteen saavuttaen sukukypsyyden ja aikuispituuden. Murrosiän ajoitukseen vaikuttaa mm. yleinen terveydentila, ravitsemus, stressi ja perimä. Murrosiän käynnistymisen ja etenemisen edellytyksenä on normaalisti toimiva hypotalamus-aivolisäke-sukupuolirauhanen-järjestelmä. Sitä, mikä varsinaisesti käynnistää murrosiän, ei kuitenkaan tiedetä. Äskettäin julkaistu havainto, että isältä peritty *MKRN3*-geenin mutaatio altistaa ennenaikaiselle murrosiälle viittaa siihen, että lapsuusiän keskushermoston estovaikutus murrosiän käynnistymiseen johtuu osin puberteettia säätelevien geenien ilmentymisestä. Tällä hetkellä vain murrosiän käynnistymisen välillisiä vaikutuksia voidaan mitata verenkierrosta, mutta käytössä ei ole merkkiainetta, joka mittaisi suoraan keskushermoston estovaikutusta murrosiän käynnistymiseen. Tällainen merkkiaine voisi auttaa viivästyneen puberteetin erotusdiagnostiikassa ja puberteetin etenemisen arvioinnissa. Monet eri sairaudet voivat viivästyttää murrosikää, mutta erityisen tärkeää olisi löytää ajoissa potilaat, joilla viivästyneen puberteetin syynä on synnynnäinen hypogonadotrooppinen hypogonadismi (HH) eli aivolisäkkeen erittämien gonadotropiinien puutteesta johtuva sukupuolirauhasten vajaatoiminta. HH:n sukupuolihormonivaje altistaa hoitamatta pitkäaikaisille haittavaikutuksille, mutta sen vaikutuksista imeväisiän ja lapsuuden kasvuun sekä terveyteen liittyvään elämänlaatuun aikuisena tiedetään hyvin vähän.

Tutkimuksen ensimmäisessä osakokonaisuudessa määritettiin seerumin MKRN3-tasot lyhytkasvuisilla pojilla puberteetin aikana. Osaa pojista hoidettiin kahden vuoden ajan aromataasin estäjä letrotsolilla aikuispituuden lisäämiseksi. Tulosten mukaan seerumin MKRN3-tasot laskevat voimakkaasti ennen murrosiän alkua sopien keskushermoston murrosiän estovaikutuksen määrän vähenemiseen. MKRN3-tasojen laskuun liittyi myös murrosiän biokemiallisten merkkiaineiden (luteinisoiva hormoni, testosteroni, inhibiini B) nousu sekä puberteetin eteneminen. Letrotsoli-hoidetuilla todettiin ennen murrosiän käynnistymistä hitaampi seerumin MKRN3-tasojen lasku kuin lumelääkettä saaneilla pojilla, viitaten siihen, että estrogeeni osallistuu murrosiän käynnistymisen säätelyyn.

Tutkimuksen toisessa osakokonaisuudessa kartoitettiin viivästyneen puberteetin syyt Helsingin yliopistollisen keskussairaalan lastenendokrinologian vastaanotolla vuosien 2004–2014 aikana. Viivästyneen murrosiän taustalta löytyi 30 eri diagnoosia, joista yleisin syy oli tuntemattomasta syystä viivästynyt murrosikä eli konstitutionaalinen viivästynyt murrosiän kehitys (CDGP). Tulokset osoittavat, että pituuskasvunopeus, aikaisemmin diagnosoitu piilokives ja lähisukulaisella todettu viivästynyt puberteetti auttavat viivästyneen murrosiän syiden erottelussa ja ennustavat murrosiän etenemistä. Lisäksi tulokset osoittavat, että synnynnäistä HH:ta sairastavilla esimurrosikäisillä pojilla kivesten koko on pienempi kuin CDGP-pojilla, ja että kivesten koon mittaaminen erottelee tehokkaasti HH:n ja CDGP:n toisistaan.

Kolmas osakokonaisuus selvitti synnynnäisen HH:n vaikutuksia imeväisiän ja lapsuusiän kasvuun. Tutkimuksessa kuvattiin *FGFR1*-geenivirhettä kantavien synnynnäistä HH:ta sairastavien tyttöjen lapsuusiän kasvun piirteet. Heidän

pituuskasvunsa oli keskimäärin ikätovereita hitaampaa 4–7 vuoden iästä alkaen jatkuen aina sukupuolihormonihoidon aloitukseen asti. Vastaavasti HH-pojilla sukupuolihormonivaje assosioitui kasvun hidastumiseen jo minipuberteetin eli ensimmäisen puolen vuoden aikana, sekä johtaa myös murrosiän kasvupyrähdyksen puuttumiseen.

Tutkimuksen neljäs osakokonaisuus käsitteli synnynnäisen sukupuolihormonivajeen vaikutusta terveyteen liittyvään elämänlaatuun aikuisiällä. HH-miehillä todettiin 15D-elämänlaatumittarilla heikentynyt elämänlaatu aikuisena. HH-potilaiden varhaista tunnistamista puoltaa tutkimuksen havainto, että HH-miehillä alentunut terveyteen liittyvä elämänlaatu assosioitui korkeampaan diagnoosi-ikään.

Tutkimustulokset osoittavat, että sukupuolihormonit säätelevät kasvua jo imeväisiästä alkaen aina murrosiän päättymiseen asti. Pojilla MKRN3-tason lasku edeltää puberteetin käynnistymistä ilmentäen todennäköisesti keskushermoston murrosiän estovaikutuksen voimakkuutta. Tulokset tukevat tämänhetkistä käsitystä, että *MKRN3*-geenillä ja estrogeenillä on tärkeä osa murrosiän käynnistymisen säätelyssä. Ne myös luovat perustaa uusien puberteetin merkkiaineiden löytymiselle ja niiden käytölle murrosiän häiriöiden tunnistamisessa. Moni sairaus hidastaa murrosiän käynnistymistä, mutta CDGP on yleisin yksittäinen syy puberteetin viivästymiseen Suomessa. HH:n tunnistaminen viivästyneen murrosiän taustalta on tärkeää, koska viivästynyt diagnoosi heikentää terveyteen liittyvää elämänlaatua jopa aikuisikään saakka. Tulokset korostavat esitietojen ja kattavan kliinisen tutkimuksen tärkeyttä selvitettäessä murrosiän viivästymisen syitä, mutta tuovat myös uusia työkaluja potilastyöhön.

INTRODUCTION

In 1977, the Nobel Prize in physiology was awarded to the scientists who discovered a small peptide, gonadotropin-releasing hormone (GnRH), secreted in brain (Burgus *et al.* 1971, Schally *et al.* 1971). The discovery of GnRH inspired further research which eventually led to the understanding of the basic functions and the genetic regulation of the hypothalamic-pituitary-gonadal (HPG) axis (Bennett *et al.* 1975, Blake *et al.* 1980, Mason *et al.* 1986, Franco *et al.* 1991, Lei & Rao 1994). Subsequently, gene mutations found in patients with congenital hypogonadotropic hypogonadism (CHH) and pubertal disorders have increased our understanding of the complex genetic control of puberty (Elks *et al.* 2010, Bianco & Kaiser 2009, Howard *et al.* 2016, Abreu *et al.* 2013). This was highlighted by the recent finding of a paternally inherited loss-of-function mutation in makorin ring finger protein 3 (MKRN3) gene in patients with central precocious puberty (CPP), which suggested that *MKRN3* has a pivotal role in the inhibition of GnRH secretion (Abreu *et al.* 2013). Measuring circulating GnRH levels has been difficult, and observing the activity of GnRH neurons has been limited to measuring its effects further downstream on the HPG axis (*i.e.* gonadotropin and sex steroid levels). However, in girls circulating MKRN3 levels have shown promising results for serving as a direct marker of central restrain on GnRH neurons, whereas similar studies in boys have not been published (Hagen *et al.* 2015).

Before puberty, the HPG axis faces two prolonged activation periods. The first occurs during fetal life and the second activation right after birth. The levels of sex steroids start to increase after the first week of life, and reach adult-like levels at 1 to 3 months of age, and then gradually decline by the age of 6 months (Forest *et al.* 1974, Winter *et al.* 1976, Bolton *et al.* 1989, Andersson *et al.* 1998, Kuiri-Hanninen *et al.* 2011, Bergada *et al.* 2006). This minipuberty has been known for over 40 years (Forest *et al.* 1974, Winter *et al.* 1976), but its importance in human development and influence on growth remains unclear. After infancy, the HPG axis remains quiescent and under central restrain until it is reactivated at the onset of puberty.

During puberty, adolescents achieve secondary sexual characteristics, experience growth spurt, and attain adult height. Thus, delayed pubertal maturation can cause low self-esteem and significant stress for adolescents and even limit their activities (Gross & Duke 1980). Delayed puberty (DP) is most commonly caused by constitutional delay of growth and puberty (CDGP) which is an idiopathic condition – an extreme end of the normal spectrum (Sedlmeyer & Palmert 2002). However, DP can also result from a variety of functional and chronic diseases. Patients with CHH have delayed or absent pubertal development, and CHH also serves as a practical model to investigate the long-term impact of sex steroid deficiency (Sedlmeyer & Palmert 2002). The differential diagnosis of DP, especially between CDGP and CHH, can be challenging (Harrington & Palmert 2012). Therefore, physicians who evaluate patients with DP in school health care or at the pediatric outpatient clinic have a crucial role in the early identification of the patients with a pathological underlying cause of DP. Consequently, understanding the diagnoses that underlie DP and the predictors of its clinical course could lead to better patient care.

The aim of this study was to evaluate if circulating MKRN3 levels could serve as a read-out of the central restrain on the HPG axis in peripubertal boys, characterize the diagnoses that underlie DP, describe the childhood growth of CHH patients, especially length growth during minipuberty, and to evaluate the long-term impact of sex steroid deficiency on health-related quality of life (HRQoL).

REVIEW OF THE LITERATURE

1. HYPOTHALAMIC-PITUITARY-GONADAL (HPG) AXIS AND PUBERTY

1.1. Current concepts on the development and function of GnRH neurons

Normally functioning HPG axis has a critical role in the reproductive development. This neuroendocrinological cascade is initiated by GnRH neurons located in the hypothalamus. However, GnRH neurons rise from the olfactory placodes where they migrate towards the forebrain and the hypothalamus (Kim *et al.* 1999, Wray 2010, Wierman, Kiseljak-Vassiliades & Tobet 2011). The sensitivity of this period is underlined by the fact that an impaired migration process results in congenital hypogonadism which clinically manifests in disorders of puberty in later life (Wray 2010, Wierman, Kiseljak-Vassiliades & Tobet 2011, Boehm *et al.* 2015, Dwyer *et al.* 2015, Young 2012, Kallmann, Schonfeld & Barrera 1944, Valdes-Socin *et al.* 2014, Legouis *et al.* 1991).

1.1.1. The ontogeny and migration of GnRH neurons

There is substantial evidence that GnRH neurons originate from the progenitor cells of the olfactory placodes of the developing embryo, whereas some have suggested that GnRH neurons arise from the neural crest (Kim *et al.* 1999, Wray 2010, Schwanzel-Fukuda & Pfaff 1989, Wray, Grant & Gainer 1989, Forni *et al.* 2011, Katoh *et al.* 2011). Nevertheless, these neurons are unique as they origin outside the central nervous system (CNS) and migrate towards the forebrain (Kim *et al.* 1999, Wray 2010, Schwanzel-Fukuda & Pfaff 1989). Based on studies in rodents, the migration pathway of GnRH neurons is marked by a variety of adhesion molecules (Bless *et al.* 2006, Garcia-Gonzalez *et al.* 2016), neurotransmitters (Casoni *et al.* 2012, Vastagh *et al.* 2015), and growth factors (Sabado *et al.* 2012, Chung & Tsai 2010). Initially, GnRH neurons attach to the axons of the vomeronasal-nerves (VNN) and climb towards the cribriform plate located at the nasal-forebrain junction. At the cribriform plate, GnRH neurons have to find their way caudally to the forebrain. Importantly, this phase reflects the close developmental relationship between the olfactory system and GnRH neurons, since an impaired GnRH migration at this stage results in a clinical syndrome of hypogonadotropic hypogonadism and anosmia (Kallmann syndrome, KS) (Boehm *et al.* 2015, Kallmann, Schonfeld & Barrera 1944, Laitinen *et al.* 2011, Hutchins *et al.* 2016, Lewkowitz-Shpuntoff *et al.* 2012). After the cribriform plate, GnRH neurons continue their pathway to the forebrain until the migration ends at the hypothalamic area. At this point, GnRH neurons detach from the guidance of the VNN (Kim *et al.* 1999, Wray 2010, Schwanzel-Fukuda & Pfaff 1989). Subsequently, the neurons form extensions towards the anterior pituitary which enable the secretion of GnRH to the hypophyseal portal blood (Wierman, Kiseljak-Vassiliades & Tobet 2011). At the anterior pituitary, GnRH regulates the secretion of gonadotropins which are critical for the normal development of gonads.

1.1.2. The composition and function of the GnRH pulse generator

Anatomically, the GnRH pulse generator consists of a complex neuronal network which regulates the pulsatile secretion of GnRH and gonadotropins (Herbison 2016, Piet *et al.* 2015). The hypothalamus receives a vast amount of intrinsic and environmental impulses. Subsequently, the information is filtered, processed, and transmitted onwards to the GnRH neurons through the adjacent neurons (**Figure 1A**). These neurons secrete neuropeptides such as kisspeptin (Herbison 2016, Ward *et al.* 2009, Millar & Newton 2013). In humans, kisspeptin secreting neurons are located in the preoptic area and the arcuate nucleus of the hypothalamus (ARC). Kisspeptin activates the G protein-coupled receptor (GPR54)/kisspeptin-1 receptor (KISS1R), and GnRH neurons are sensitive to kisspeptin and express *KISS1R* (Pinilla *et al.* 2012). In mice, the amount of kisspeptin sensitive GnRH neurons increase during peripuberty (Kallo *et al.* 2012). Kisspeptin stimulates GnRH secretion, and the administration of a kisspeptin analog to prepubertal mice induces the progression of puberty (Decourt *et al.* 2016). Conversely, an inactivating mutation in *Kiss1* results in hypogonadism marked by the absence of puberty (Seminara *et al.* 2003). In humans, a loss-of-function mutation in *KISS1* is reported to cause CHH (Seminara *et al.* 2003), whereas activating mutations in *KISS1* have been found in patients with CPP (Silveira *et al.* 2010). Most of the kisspeptin neurons secrete two co-transmitters: dynorphin (Dyn) and neurokinin B (NKB) (Skrapits *et al.* 2015). NKB and its receptor are encoded by *tachykinin-3* (*TAC3*) and its receptor (*TACR3*), respectively. In mice, an NKB analog induces GnRH activity and LH pulses through kisspeptin/gpr54 signaling (Grachev *et al.* 2012). The administration of NKB alone does not appear to provoke gonadotropin secretion in humans, whereas administering it with kisspeptin increases LH secretion (Narayanaswamy *et al.* 2016). In support of the view that NKB stimulates GnRH secretion, a loss-of-function mutation in *TAC3* or *TACR3* results in congenital hypogonadotropism in humans (Topaloglu *et al.* 2012). Dynorphin synchronize and modulate the secretion of kisspeptin and transmit inhibitory signals to the GnRH neurons, thus in Dyn knockout mice gonadotropin secretion is compromised (Navarro *et al.* 2009). A subpopulation of kisspeptin neurons co-secrete kisspeptin, NKB, and DYN, and are located in the ARC (Skrapits *et al.* 2015). These neurons are called KNDy neurons, and they regulate the GnRH neurons (**Figure 1A**) (Pinilla *et al.* 2012). Gamma-amino butyric acid (GABA) influences on GnRH neurons through $GABA_A$ receptors (Watanabe, Fukuda & Nabekura 2014). Despite being an inhibitory neurotransmitter in the adult brain, *in vivo* studies suggest that GABA mediates excitatory impulses to GnRH neurons (Watanabe, Fukuda & Nabekura 2014). All in all, the intensity of transmitted signals to GnRH neurons determines whether the GnRH secretion is maintained at the basal amplitude or if the information is transmitted onwards in a form of altered GnRH secretion.

GnRH is released into the pituitary portal blood with a diurnal pattern, particularly during the puberty onset (Rosenfield, Bordini & Yu 2013, Rosenfield, Bordini & Yu 2012, Albertsson-Wikland *et al.* 1997). An increase in GnRH secretion stimulates the anterior pituitary to secrete gonadotropins (*i.e*. luteinizing hormone (LH) and follicle-stimulating hormone (FSH)). In women, LH is required in the induction of ovulation, and FSH for the maturation of the follicles in the ovaries (Simonneaux & Bahougne 2015). In men, FSH acts directly on the Sertoli cells, whereas LH stimulates androgen production in Leydig cells to promote spermatogenesis (O'Shaughnessy 2014, Huhtaniemi 2015). Additionally, the

testes secrete inhibin B which inhibits FSH secretion at the pituitary level (de Kretser *et al.* 2000, Andersson *et al.* 2004, Boepple *et al.* 2008, Sehested *et al.* 2000), and anti-müllerian hormone (AMH) which is crucial for the development of sexual dimorphism as it induces the atrophy of the Müllerian ducts in male fetuses (Josso, Picard & Tran 1980, Josso, Rey & Picard 2013) (**Figure 1A**).

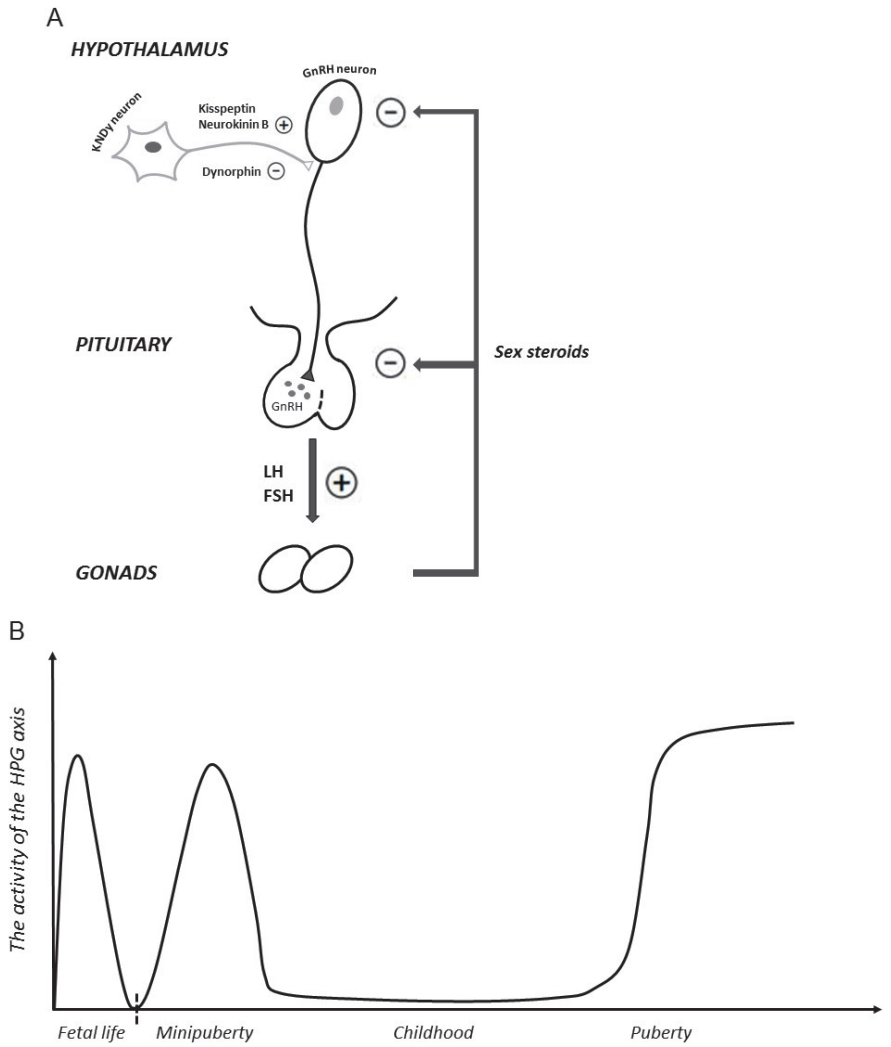

Figure 1. *Panel A*, The negative-feedback loop of the hypothalamic-pituitary-gonadal (HPG) axis after puberty. Gonadotropin-releasing hormone (GnRH) neurons secrete GnRH into the portal blood of anterior pituitary, which induces the secretion of gonadotropins. Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) induce sex steroid production in the gonads. Sex steroids, especially estrogen, inhibit GnRH and gonadotropin secretion. At least a part of the feedback on GnRH neurons is mediated through kisspeptin, neurokinin B, and dynorphin. *Panel B*, the activity of the HPG axis from fetal life to adolescence (Pinilla *et al.* 2012, de Kretser *et al.* 2000, Kuiri-Hanninen, Sankilampi & Dunkel 2014, Jin & Yang 2014).

1.1.3. The activation phases of the HPG axis

Before puberty, the HPG axis faces two activation periods (**Figure 1B**). The first takes place during fetal life when the levels of LH and FSH peak at the mid-gestation (Kaplan & Grumbach 1976, Debieve *et al.* 2000). This hormonal surge is more pronounced in the female than in the male fetuses (Reyes, Winter & Faiman 1973, Clements *et al.* 1976). Thereafter, the levels of LH and FSH decline reaching only low levels at birth (Debieve *et al.* 2000). The second activation period occurs after birth. During the first 24 hours of life, there is a transient peak in LH secretion which resolves quickly (Corbier *et al.* 1990). Then, the HPG axis is reactivated at the age of one week and the active phase lasts for the next 6 to 9 months (Forest *et al.* 1974, Winter *et al.* 1976, Kuiri-Hanninen, Sankilampi & Dunkel 2014). During this minipuberty, gonadotropin and sex steroid levels increase and reach adult-like levels at 1 to 3 months of age (Winter *et al.* 1976, Tapanainen *et al.* 1981, Andersson *et al.* 1998, Bergada *et al.* 2006, Kuiri-Hanninen, Sankilampi & Dunkel 2014), and serum inhibin B and AMH levels surge concomitantly (Andersson *et al.* 1998, Aksglaede *et al.* 2010). However, the postnatal surge in hormonal levels differs between sexes. In boys, the gonadotropin response is LH dominant, whereas in girls FSH is more profoundly elevated (Bergada *et al.* 2006, Ibanez *et al.* 2002). Testosterone levels decline by 6 months of age, but girls may still show elevated estradiol levels at 3-4 yrs of age (Kuiri-Hanninen *et al.* 2011).

Estradiol levels fluctuate, whereas testosterone levels peak more sharply. Besides hormonal changes, maturing follicles can be detected in girls during minipuberty (Kuiri-Hanninen *et al.* 2013). In primates testis, the number of Sertoli cells increase from birth to infancy concomitantly with the elevated gonadotropin levels (Simorangkir, Marshall & Plant 2003), which suggests that minipuberty is important for the proliferation of Sertoli cells (Simorangkir, Marshall & Plant 2003). In support of this, boys with CHH may show normal genitalia at birth, but a progressive involution of the scrotum during minipuberty (Main, Schmidt & Skakkebaek 2000). At the same time, the number of germ cells, Sertoli cells and Leydig cells in healthy boys increase, and urinary prostate specific antigen levels are transiently elevated (Kuiri-Hanninen *et al.* 2011, Codesal *et al.* 1990, Muller & Skakkebaek 1984, Cortes, Muller & Skakkebaek 1987). However, the initiation of spermatogenesis is prevented by the absence of androgen receptors in Sertoli cells (Chemes *et al.* 2008). During the first six months, testicular and penis size increase (Kuiri-Hanninen *et al.* 2011, Boas *et al.* 2006). The hormonal minipuberty has been known for 40 years, but only recently some of its importance in human development has been understood. The early hormonal surge, especially testosterone, is associated with male-type behavior in both boys and girls (Lamminmaki *et al.* 2012). Low testosterone levels are associated with early phonological development and language organization in both sexes (Friederici *et al.* 2008, Quast *et al.* 2016).

During childhood, the HPG axis remains quiescent under the central restrain with the exception of some overnight LH secretion (Mitamura *et al.* 2000, Mitamura *et al.* 1999, Apter *et al.* 1993, Wu *et al.* 1996). Despite of the gonadostat, the HPG axis remains functional since it can be evoked with a GnRH test which in prepubertal patients shows a small but a significant increase in LH levels and a considerable FSH response (Dickerman, Prager-Lewin & Laron 1979). The onset of puberty is preceded by the removal of the gonadostat which results in an increased amplitude and frequency of GnRH secretion (Herbison 2016, Oerter *et*

al. 1990). Subsequently, the levels of gonadotropins increase and stimulate sex steroids secretion which ultimately induce pubertal maturation (Oerter *et al.* 1990, Andersson *et al.* 1997).

1.1.4. The regulation of the HPG axis

After the onset of puberty the HPG axis is under the regulation of a negative-feedback loop (**Figure 1A**). The role of estrogen in regulating GnRH secretion appears to be bipartite. Estrogen inhibits *Kiss1* expression and gonadotropin secretion in mice, but there is evidence of a positive estrogen-kisspeptin feedback circuit as well (Dorling *et al.* 2003, Clarkson *et al.* 2010, Dubois *et al.* 2015, Dubois *et al.* 2016). In pubertal primates, the removal of circulating estrogen increases the secretion of kisspeptin, and the administration of estrogen restores the kisspeptin activity suggesting that estrogen is a crucial inhibitor of GnRH secretion (Kenealy *et al.* 2016, Kenealy *et al.* 2013). Testosterone also appear to regulate GnRH secretion, since a treatment with testosterone in castrated male monkeys suppresses LH and FSH secretion and the levels of kisspeptin mRNA in mediobasal hypothalamus (Shibata *et al.* 2007). Due to the aromatization of androgens into estrogens, the inhibitory effects of testosterone on the HPG axis are believed to be estrogen-mediated. The administration of estrogen decreases the LH secretion in both healthy men and men with isolated GnRH deficiency (Finkelstein *et al.* 1991), whereas the removal of the estrogen inhibition with an aromatase inhibitor (AI), which is a blocker of estrogen biosynthesis, results in increased levels of gonadotropins (Finkelstein *et al.* 1991, Hayes *et al.* 2000, Wickman & Dunkel 2001). Moreover, in late maturing boys who were treated simultaneously with an AI and testosterone, the levels of baseline gonadotropins and GnRH induced LH-response increased significantly, which further supports the key role of estrogen in the regulation of human puberty (Wickman & Dunkel 2001).

1.2. Normal puberty

1.2.1. General concepts

During puberty, the adolescent achieves secondary sexual characteristics and reproductive capability, and eventually attains adult height. During the last century, the timing of puberty has declined rapidly until the 1950s, and, thereafter, the decrease has shown a steadier phase (Euling *et al.* 2008). The onset of puberty is marked by the development of glandular breast tissue in girls and an increase in testicular volume in boys (Marshall & Tanner 1969, Marshall & Tanner 1970). Timing of puberty shows large individual variation. Puberty may begin too early or it can be delayed. In Finland, all adolescents are screened for abnormal pubertal maturation at the age of 13 to 14 yrs in the school health care.

1.2.2. Normal variation in the timing of puberty and secular trends

Puberty onset normally occurs in boys at the average age of 11 to 12 yrs, but can vary from 9 to 14 yrs (Marshall & Tanner 1970, Ojajärvi 1982), whereas in girls, puberty begins at an average age of 10 to 11 yrs, but is still considered normal if glandular breast tissue (Tanner B2 starge) is found between 8 to 13 yrs of age (Marshall & Tanner 1969, Ojajärvi 1982). Another difference between the sexes

is observed in bone maturation. The onset of puberty occurs at a mean bone age of 11.1 years in girls and 11.7 years in boys (Marshall 1974).

The decline in the age of pubertal timing has been biphasic. The age of menarche decreased gradually until the 1950s, and then the decline has plateaued as a result of better nutrition, hygiene, and diet (Sorensen *et al.* 2012). During the last two decades, several studies have investigated pubertal timing, and a trend towards an earlier puberty in girls has been reported both in the US and Europe (Sun *et al.* 2002, Aksglaede *et al.* 2009, Biro *et al.* 2013). However, the results of the studies are inconsistent. Studies over longer period of time failed to show a decline in the age of puberty onset during the last 20 yrs, which suggested a difference in the timing of puberty between the US and Europe (Juul *et al.* 2006, Mul *et al.* 2001). The Copenhagen puberty study reported a decline in the age of thelarche from 10.9 to 9.9 yrs during a period of 15 yrs, but the decrease in the age of thelarche was not as significant as reported in the US (Sun *et al.* 2002, Aksglaede *et al.* 2009, Wu, Mendola & Buck 2002). Further, the decline in the timing of puberty was not supported by a change in the biochemical markers of puberty (Aksglaede *et al.* 2009). The levels of gonadotropins and estrogen did not increase in parallel to the decrease in the age of thelarche, which pointed towards the earlier appearance of glandular breast tissues independent of gonadotropin secretion (Aksglaede *et al.* 2009). The age at menarche appears to decline. A recent study reported a trend towards an earlier age of thelarche, and also an earlier menarche in daughters than their mothers (Wohlfahrt-Veje *et al.* 2016). The age at menarche in mothers correlate with the age at menarche in daughters, and similarly the pubertal timing of mothers and fathers influence on the age of puberty onset of their sons and daughters (Wohlfahrt-Veje *et al.* 2016, Ersoy *et al.* 2005). Thus, the timing of puberty appears to be parentally inherited.

1.2.3. Clinical and biochemical markers of puberty

Pubertal progress is categorized by using the Tanner stage classification which divides the development of the testes, breasts, and pubic hair into five categories (Marshall & Tanner 1969, Marshall & Tanner 1970). In boys, prepubertal genitals are categorized into genital stage 1 (G1), and when puberty is complete, the genital stage is 5 (G5) (Marshall & Tanner 1970). Similarly, in girls, the pubertal progression is classified from breast stage 1 to 5 (B1-B5) (Marshall & Tanner 1969).

In boys, a testis size more than 3 mL is widely used to indicate the clinical onset of puberty, whereas some studies have suggested that testicular volume of 4 mL marks the activation of the HPG axis (Sadov *et al.* 2016, Joustra *et al.* 2015, Ankarberg-Lindgren & Norjavaara 2004). Correspondingly, the appearance of glandular breast tissue (thelarche) (B2) indicates the clinical onset of puberty in girls (Marshall & Tanner 1969, Ojajärvi 1982).

Tanner genital stage is a simple and the most widely used clinical assessment of the clinical onset and progression of puberty. However, it relies solely on the visual estimate of the breast, the genital, and the pubic hair stage. In boys, measuring the length and the width of the testes is an accurate method to estimate testicular volume. Several formulas have been used to estimate testis size, one of which is the Hansen formula: (mL) is length (cm) x width^2 (cm) x 0.52 (Behre, Nashan & Nieschlag 1989). Another method used widely in clinical practice to determine testis size is the Prader orchidometry (Prader 1966). Testicular size can

be also measured with ultrasound (Diamond *et al.* 2000), but its usefulness is limited by costs and availability. Additionally, none of the three measuring methods is superior to the others in accuracy (Taskinen, Taavitsainen & Wikstrom 1996). In girls, evaluating the presence of breast tissue can be very difficult, especially in an obese subject. In unclear cases, breast tissues can be determined by using the ultrasound (Garcia *et al.* 2000).

Biochemical markers of puberty illustrate the activity of the HPG axis at the pituitary and at the gonadal level. A single measurement of LH or FSH represent indirectly the GnRH secretion. Additionally, the activity of the HPG axis can be assessed with two stress-tests. In a human chorionic gonadotropin (hCG) test, gonadal testosterone secretion is stimulated with hCG (Segal *et al.* 2009, Osuna, Arata-Bellabarba & Tortolero 2001), whereas in a GnRH test, LH and FSH releases are augmented by administering a GnRH analog (Rosenfield, Bordini & Yu 2013, Rosenfield, Bordini & Yu 2012, Dunkel *et al.* 1985). At gonadal level, the Sertoli cells inside the seminiferous tubules of the testes secrete inhibin B which can be used in the biochemical evaluation of puberty. However, the measurement of low inhibin B levels is subject to a high variation (Kalra *et al.* 2010). Another Sertoli cell origin peptide, AMH, has been reported in studies addressing pubertal disorders. Serum AMH levels declined during puberty which reflected the gonadotropin influence on the Sertoli cell maturation (Hero *et al.* 2012). Moreover, inhibin B and AMH levels have been suggested to discriminate between the boys with idiopathic pubertal delay and CHH (Coutant *et al.* 2010, Adan *et al.* 2010).

1.2.4. Molecular genetic basis of puberty

During the last 30 yrs, the discovery of novel genes in patients with pubertal disorders, especially CHH, has revealed the genetic complexity of human puberty (Elks *et al.* 2010, Bianco & Kaiser 2009, Boehm *et al.* 2015). In the past, the genes associated with CHH were discovered by identifying single candidate CHH genes and linking them to the phenotypic features of CHH (Pitteloud *et al.* 2006, Dode *et al.* 2003, Dode *et al.* 2006). Additionally, depicting the pedigrees of CHH patients has been fruitful in identifying new candidate genes and revealing the low penetrance for most of the CHH genes (Gurbuz *et al.* 2012). By using these methods approximately 30 to 35% of genes that underlie CHH have been discovered (Crowley 2011), which suggests that many patients with CHH are currently without a genetic diagnosis. Recently, new methods such as next-generation sequencing, proteomics, and genomics have yielded additional precision in finding novel CHH genes. The problem in identifying causative CHH genes lies in the absence of consensus of validation standards (MacArthur *et al.* 2014). However, whole-genome sequencing and its implications are likely to reveal the remaining portion of unknown CHH genes in the near future (Stamou, Cox & Crowley 2015).

Very recent discoveries of genes that underlie delayed and precocious puberty have increased our knowledge on the genetic control of human puberty. A loss-of-function mutation in *MKRN3* was discovered in patients with CPP (Abreu *et al.* 2013). Subsequently, circulating MKRN3 levels were measured in girls before and after the onset of puberty (Hagen *et al.* 2015). These findings suggested that a normally functioning *MKRN3* is critical for maintaining the central inhibition on the GnRH neurons. *MKRN3* is expressed widely in human tissues, and is a putative E3 ubiquitin ligase, but its exact mechanisms are unclear (reviewed in Abreu *et*

al. 2015). Very recently, a pathologic mutation in *immunoglobulin superfamily member 10* (*IGSF10*) gene was found in six unrelated Finnish families with late puberty (Howard *et al.* 2016). Apparently, these genes control the timing of puberty, but, at the same time, they represent only a fraction of potential puberty genes that are yet to be found.

The knowledge of genetic regulation of puberty stems mainly from the mutations found in patients with CHH. The most common genes that are found mutated in CHH patients, especially the ones reported in Finnish patients, are listed in Table 1 (**Table 1**). In brief, CHH genes regulate GnRH neuron migration and development or GnRH secretion and action (**Table 1**). The inheritance pattern differs between CHH genes, for example, *anosmin 1* (*ANOS1*) is located in chromosome X, thus mutations in *ANOS1* manifest commonly in male CHH patients (Boehm *et al.* 2015). Remarkably, only less than 10% of CHH cases have a molecular genetic verification of CHH (Boehm *et al.* 2015). In Finland, the most common mutated CHH gene is *fibroblast growth factor receptor 1* (*FGFR1*). In fact, one third of the Finnish patients with CHH carry a mutation in *FGRF1* (Laitinen *et al.* 2011). Further, *FGFR1* mutations manifest with skeletal anomalies and, at a population level, are suggested to be associated with childhood obesity (Jiao *et al.* 2011, Costa-Barbosa *et al.* 2013), whereas *ANOS1* associates with a more severe reproductive phenotype of CHH (Costa-Barbosa *et al.* 2013).

Table 1. Phenotypes and non-reproductive features of the most common genes found mutated in patients with Kallmann syndrome (KS), congenital hypogonadotropic hypogonadism (CHH), constitutional delay of growth and puberty (CDGP) and central precocious puberty (CPP).

Gene	HGNC ID	Phenotypes					Reported in Finnish patients	Mode of inheritance	Distinctive non-reproductive features	Reference(s)
		KS	CHH	CHH reversal	CDGP	CPP				
Development and migration of GnRH neurons										
ANOS1	HGNC:6211	x		x			x	X-linked	Anosmia	(Laitinen *et al.* 2011, Costa-Barbosa *et al.* 2013)
FGFR1	HGNC:3688	x	x	x			x	Autosomal dominant	Bony anomalies	(Laitinen *et al.* 2011, Costa-Barbosa *et al.* 2013)
FGF8	HGNC:3686	x	x					Autosomal dominant	Dental agenesis	(Costa-Barbosa *et al.* 2013)
PROK2	HGNC:18455	x	x					Autosomal resessive		(Dode *et al.* 2006)
PROKR2	HGNC:15836	x	x	x			x	Autosomal resessive		(Tommiska *et al.* 2013)
CHD7	HGNC:20626	x	x	x				Autosomal dominant	CHARGE, hearing loss	(Costa-Barbosa *et al.* 2013)
SEMA3A	HGNC:10723	x					x			(Boehm *et al.* 2015)
SEMA7A	HGNC:10741	x					x			(Boehm *et al.* 2015)
SOX10	HGNC:11190	x					x	Autosomal dominant	Hearing loss	(Vaaralahti *et al.* 2014, Pingault *et al.* 2013)
IGSF10	HGNC:26384				x		x			(Howard *et al.* 2016)
GnRH secretion and action										
GNRHR	HGNC:4421		x	x	x		x	Autosomal resessive		(Vaaralahti *et al.* 2011, Laitinen *et al.* 2012b)
TAC3	HGNC:11521		x	x				Autosomal resessive		(Gianetti *et al.* 2010)
TACR3	HGNC:11528		x	x				Autosomal resessive		(Gianetti *et al.* 2010)
KISS1	HGNC:6341		x							(Topaloglu *et al.* 2012)
KISS1R	HGNC:4510		x							(de Roux *et al.* 2003)
Inhibition of GnRH secretion										
MKRN3	HGNC:7714					x		Autosomal dominant		(Abreu *et al.* 2013)

1.3. Delayed puberty (DP)

The majority of adolescents enter puberty within the normal age range. However, sometimes pubertal maturation occurs later than expected. Delayed puberty is defined as the absence of clinical signs of puberty at the age of 2 to 2.5 standard deviation (SD) above the mean age of the general population, and it affects approximately 2% of adolescents (Marshall & Tanner 1969, Marshall & Tanner 1970, Lee 1980). Delayed puberty is caused by a variety of conditions (Sedlmeyer & Palmert 2002, Reindollar & McDonough 1981, Lawaetz *et al.* 2015). The most common cause is constitutional delay of growth and puberty (CDGP) which constitutes up to 65% of cases with DP at a single pediatric endocrinology outpatient clinic (Sedlmeyer & Palmert 2002). On the other hand, DP can be caused by chronic diseases and pathological entities, such as craniopharyngioma and Crohn's disease, which need to be diagnosed and treated as early as possible. This emphasizes the key role of first-line evaluation in identifying these patients early in school health care and referring them onwards to the pediatric outpatient clinic.

1.3.1. Diagnostic criteria in Finland

Due to the secular trend in the timing of puberty, the diagnostic criteria of DP show variation between countries. Since two different testicular sizes have used to describe the clinical onset of puberty, the diagnostic criteria are not uniform either. In Finland, the age limits for precocious and delayed puberty was recently updated by an expert consensus (Raivio 2013). Consequently, a boy is considered to have DP if he is prepubertal (*i.e.* the pubertal stage G1 and/or testis volume less than 3 mL) at the age of 13.5–14 yrs (Ojajärvi 1982), and in a girl if she is at the B1 stage at the age of 13.0 or more (Ojajärvi 1982).

1.3.2. Underlying causes

The most frequent cause of DP is CDGP which is an extreme end of normal variation (Sedlmeyer & Palmert 2002, Clayton *et al.* 1988). Thus, CDGP is an idiopathic condition which is often inherited, since up to 65% of the first degree relatives of CDGP patients report a history of DP (Sedlmeyer & Palmert 2002). However, a family history of DP is not pathognomonic to CDGP as late maturation is also found among the close relatives of patients with CHH (Sedlmeyer & Palmert 2002). The diagnosis of CDGP is set after the pathological causes of DP are ruled out. The other diagnoses can be classified into three categories. Patients with permanent hypogonadotropic hypogonadism (PHH) have low gonadotropin and sex steroid levels that result from a pathology in the CNS or a permanent disease that delays puberty. Importantly, CHH constitutes the majority of cases with PHH (Sedlmeyer & Palmert 2002). Similarly, functional hypogonadotropic hypogonadism (FHH) category consists of patients who have low gonadotropin and sex steroid levels but do not have any biochemical and clinical signs that would suggest the presence of a permanent cause of DP and an identified disease, such as celiac disease that is likely to delay maturation (Sedlmeyer & Palmert 2002). Finally, high basal gonadotropin levels and gonadal failure is characteristic to patients with hypergonadotropic hypogonadism (Hyper H). The frequency and distribution of the four diagnostic categories of DP at a pediatric outpatient clinic of a single tertiary center is presented in Figure 4 (**Figure 2**).

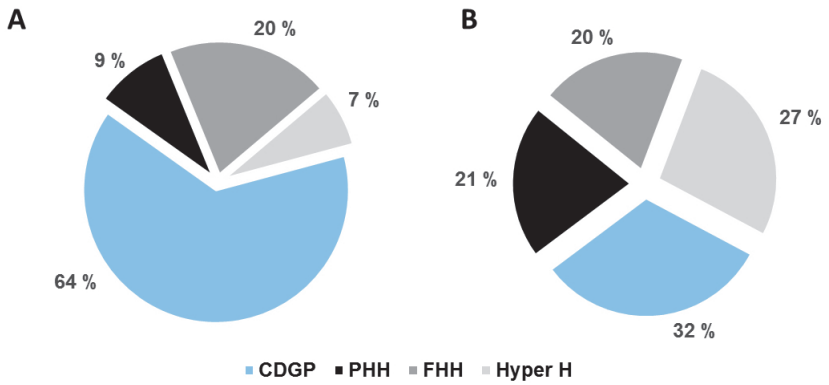

Figure 2. The frequency of causes that underlie delayed puberty in boys (A) and in girls (B) evaluated in a single tertiary center. Modified from Sedlmeyer *et al.* (Sedlmeyer & Palmert 2002). CDGP, Constitutional delay of growth and puberty; PHH, permanent hypogonadotropic hypogonadism; FHH, functional hypogonadotropic hypogonadism; Hyper H, hypergonadotropic hypogonadism.

1.3.3. Evaluation of patient with DP

The evaluation of DP includes a careful clinical examination and a review of the medical history. A history of undescended testes (*i.e.* cryptorchidism), micropenis, anosmia/hyposmia, and prior infertility treatments in close relatives are clinical cues that increase the risk for permanent hypogonadism (reviewed in Bouvattier *et al.* 2011). History of headache and other neuro-ophthalmic symptoms should be screened as well (Taylor *et al.* 2012). Subsequently, adolescents who present with the above medical history or signs and symptoms that raise a suspicion of pathologic cause of DP should be referred to the pediatric outpatient clinic for further investigation.

The diagnostic process of DP is categorized into the first and second-line investigations (Palmert & Dunkel 2012). The first-line investigations include the evaluation of genital and pubic hair stage, determining the annual growth velocity and bone age, measuring baseline sex steroid and gonadotropin levels, and biochemical analyses (e.g. thyrotropin, free thyroxine, and creatine) to rule out chronic diseases such as hypothyroidism or celiac disease (Palmert & Dunkel 2012). High basal LH and FSH levels easily identify patients with Hyper H (Abitbol, Zborovski & Palmert 2016). Sex steroid levels can be used in predicting the progress of puberty. In a previous study, 75% of boys with testosterone level above 0.7 nmol/L entered puberty within the next 12 months, and everyone within the next 15 months (Wu *et al.* 1993). Gonadotropin, estradiol, and testosterone levels show diurnal variation during puberty. After the onset of puberty, LH secretion increases during the day, whereas the diurnal secretion of FSH is less prominent (Albertsson-Wikland *et al.* 1997, Mitamura *et al.* 1999, Goji & Tanikaze 1993). Testosterone secretion is induced during the night before the clinical onset of puberty, and thereafter the levels of testosterone increase both during day and night (Albertsson-Wikland *et al.* 1997, Mitamura *et al.* 1999, Goji & Tanikaze 1993). The circadian rhythm of the estradiol is similar to that of testosterone (Mitamura *et al.* 2000).

During puberty, the gradual increase in sex steroid levels, especially estrogen, induces skeletal maturation. A delay in the onset of puberty results in a deviation also in the bone age. Thus, a patient with DP has a bone age which is approximately 2 to 3 yrs behind the calendar age (Lawaetz *et al.* 2015). Additionally, the absence of sex steroid-induced growth spurt can be visualized in the growth curve (**Figure 3**). The adolescents with DP continue to grow with the childhood growth velocity, whereas the height velocity of their peers rapidly increases. This results in a "dip" seen in the growth curve (**Figure 3**). Importantly, being shorter than their peers may be one of the reasons why patients with DP, especially boys, suffer from psychosocial burden (Gross & Duke 1980, Palmert & Dunkel 2012). Half of the boys with CDGP experience a decrease in height velocity already during childhood (Wehkalampi *et al.* 2007). In adolescence, a very slow height velocity (< 2 cm/yr), especially in a combination with neuro-ophthalmic symptoms, increases the risk for chronic diseases and panhypopituitarism (Taylor *et al.* 2012). However, only limited evidence of height velocity's discrimination between the four diagnostic categories of DP exists (*i.e*. CDGP, PHH, FHH, and Hyper H) (Palmert & Dunkel 2012).

In some cases, the first-line investigations of DP cannot provide sufficient information to rule out the pathological causes of DP. Thus, the second-line investigations offer more precise information about the HPG axis. The studies include GnRH and hCG stress testing, a brain MRI, and the markers of Sertoli cell function, and genetic testing (reviewed in Palmert & Dunkel 2012). The performance of inhibin B and AMH together with GnRH and hCG tests in the differential diagnosis of DP (*i.e.* CDGP *vs* CHH) is discussed in detail below. A brain MRI scan is performed in cases where the clinician suspects that pubertal delay might be caused by CHH, or a lesion in the CNS, or requires the visualization of the olfactory or inner ear structures (*i.e.* suspicion of CHH) (Lewkowitz-Shpuntoff *et al.* 2012).

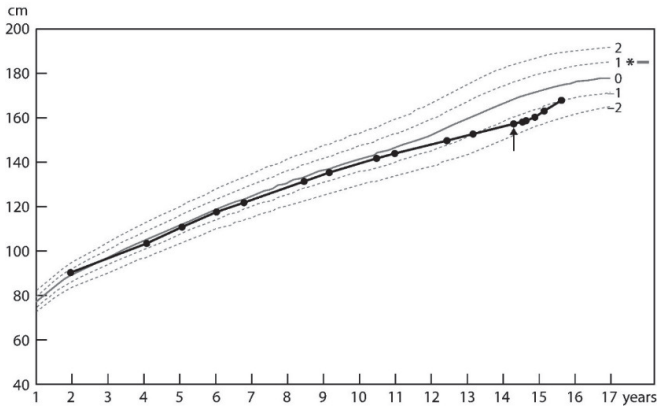

Figure 3. A typical growth curve, mid-parental target height (MPH) (asterisk), and predicted adult height (vertical line) of a boy with constitutional delay of growth and puberty (CDGP) who received testosterone treatment (1 mg/kg) (arrow) for 6 months.

1.3.4. Treatment

Before hormonal induction of puberty, the pathologic causes of DP must be excluded with a sufficient probability (Palmert & Dunkel 2012). The patients with CDGP have two treatment options: sex steroid treatment or watchful waiting (Dunkel & Quinton 2014). The onset of puberty can be waited with a careful follow-up (Dunkel & Quinton 2014). However, the absence of puberty can cause significant psychosocial stress for the adolescent (Gross & Duke 1980), which supports the induction of puberty (Palmert & Dunkel 2012). The treatment of DP (girls with estrogen gel and boys with low-dose intramuscular testosterone in Finland) intends to induce sexual maturation and an increased growth velocity, and appears to affect positively on self-esteem (**Figure 3**) (Soliman, Khadir & Asfour 1995, Bergada & Bergada 1995, Kaplowitz 1998).

Usually, estrogen gel is administered with gradually increasing amounts, and the response is evaluated at the follow-up visits (Wehkalampi *et al.* 2011, Ankarberg-Lindgren, Kristrom & Norjavaara 2014). The concept of starting the treatment with low estrogen doses stem from the idea of mimicking the normal puberty. Indeed, at early puberty low estrogen levels promote growth, whereas a higher level that occurs late in puberty is required to complete the physical development (Albin *et al.* 2012). Low-dose testosterone treatment is well-tolerated and has relatively few adverse effects, but the dosage and the treatment period vary within and between countries (Palmert & Dunkel 2012, Ambler 2009). Ideally, the hormonal treatment of DP should result in the activation of the HPG axis. Currently, this may not be the case with boys who are treated with intra muscular testosterone, because low-dose testosterone treatment suppresses gonadotropin and testosterone secretion (Soliman, Khadir & Asfour 1995, Dunkel & Wickman 2001). Testosterone is aromatized into estrogens which mediate the negative feedback to the hypothalamic and the pituitary levels of the HPG axis (**Figure 1A**) (Dunkel & Wickman 2001). Removing the negative feedback of estrogen is a potential novel treatment modality for DP (reviewed in Palmert & Dunkel 2012). Aromatase inhibitor letrozole increases gonadotropin and testosterone levels during peripuberty (Hero, Norjavaara & Dunkel 2005), which suggests that it also augments the progression of puberty. On the other hand, the impact of AIs on bone health remains unclear (Hero *et al.* 2010, Hero 2016). A well-designed randomized controlled study on the use of AI for the treatment of DP is definitely required (Palmert & Dunkel 2012). The treatment of DP should always include a follow-up to ensure the normal progression of puberty, and the absence or deceleration of the tempo of puberty and growth during the follow-up should raise the suspicion of a pathologic cause of DP, even though the initial diagnostic work-up resulted in the diagnosis of CDGP.

1.4. Congenital hypogonadotropic hypogonadism (CHH)

Congenital hypogonadotropic hypogonadism is a rare reproductive disorder which is caused by an impaired secretion, production, or action of GnRH (Boehm *et al.* 2015, de Roux *et al.* 1997, Bouligand *et al.* 2009). When hypogonadotropism is accompanied by anosmia or hyposmia, it is called Kallmann syndrome (KS). KS affects approximately 1:48000 live births in Finland (Laitinen *et al.* 2011). The patients with most severe CHH suffer from sex steroid deficiency throughout their life, starting from fetal life when the deficiency may manifest in cryptorchidism or micropenis, and result in an absent or incomplete puberty in adolescence (Main,

Schmidt & Skakkebaek 2000, Grumbach 2005, Dwyer, Jayasena & Quinton 2016). In some cases, patients with CHH may experience a reversal of hypogonadism later in life. In fact, the reversal variants constitute up to 18% of CHH cases, and the reversal of hypogonadism occurs despite of partial or absent puberty (Raivio *et al.* 2007, Sidhoum *et al.* 2014). The objectives in treating a CHH patient are to achieve secondary sexual characteristics and fertility, and induce linear growth and bone maturation (Boehm *et al.* 2015). The long-term health effects of CHH include an increased risk for osteoporosis (Laitinen *et al.* 2012a, Behre *et al.* 1997), metabolic diseases (Zarotsky *et al.* 2014), and impaired health-related quality of life (Aydogan *et al.* 2012, Shiraishi, Oka & Matsuyama 2014). Consequently, this underpins the importance of the appropriate timing of diagnosis and early treatment initiation.

1.4.1. Diagnostic criteria

The classical diagnostic criteria of CHH include an absent or incomplete puberty at the age of 18 years, low sex steroid levels with normal or subnormal gonadotropin levels, and otherwise normal anterior pituitary function, and no organic cause for the condition (Raivio *et al.* 2009, Villanueva *et al.* 2015, Shaw *et al.* 2011). Thus, excluding other causes of permanent hypogonadism, such as craniopharyngioma, is essential for the diagnosis of CHH. Although these diagnostic criteria are widely used, there is a lack of international diagnostic criteria for CHH (Boehm *et al.* 2015).

1.4.2. Clinical and biochemical evaluation

The diagnosis of CHH is typically made during late adolescence or early adulthood. To discriminate patients with CHH from those with CDGP before puberty may be extremely difficult, and diagnosing CHH without genital anomalies during childhood or infancy is even more challenging (Dwyer, Jayasena & Quinton 2016). The same clinical and biochemical markers as in the evaluation of patients with DP are used in the diagnostic process of CHH. Additionally, a brain MRI is used to exclude lesions in the CNS and to visualize the olfactory bulbs and the inner ear (Della Valle *et al.* 2013). Ultrasonography of abdomen and pelvis can reveal renal agenesis and visualize the reproductive organs (ovaries, uterus, and testis). Since sex steroids are critical for normal pubertal growth spurt and the accumulation of bone mineral content, patients with CHH have an increased risk for osteoporosis (Laitinen *et al.* 2012a). The bone density is typically assessed with the dual-energy X-ray absorptiometry (DXA).

The recent advances in molecular genetic testing have implemented the genetic testing of CHH into the diagnostic process of CHH. Molecular genetic testing is performed in order to find the inheritance pattern and the penetrance of the disease in the family (reviewed in Boehm *et al.* 2015, Pitteloud *et al.* 2010). Non-reproductive phenotype may be used in selecting patients to be screened for CHH genes. In fact, bony anomalies and dental agenesia are enriched in CHH patients with *FGFR1* mutations (Costa-Barbosa *et al.* 2013, Zenaty *et al.* 2006), whereas mutations on *ANOS1* are associated with a more severe reproductive phenotype and an increased prevalence of synkinesia (*i.e.* mirror movements) of the upper extremities (Salenave *et al.* 2008). In Finnish CHH patients, an algorithm which uses the non-reproductive phenotype can be used to guide the molecular genetic testing (Laitinen 2012). Genetic testing provides additional

information to the clinical practice, but it should be focused on the selected patients with CHH (Boehm *et al.* 2015).

The reversal CHH variants manifest in a broad spectrum of pubertal progress and non-reproductive phenotypes (Raivio *et al.* 2007). Testicular growth during follow-up may reveal the partial gonadotropin secretion seen in the reversal phenotype, but, again, differentiating these subjects from patients with CDGP is challenging (Raivio *et al.* 2007). Recently, a study of Chan *et al.* suggested that administration of kisspeptin could aid in identifying patients with a reversal form of CHH (Chan *et al.* 2014). Patients with CHH, except reversal phenotypes, did not present an LH response to administered kisspeptin (Chan *et al.* 2014). Consequently, an experiment of treatment pause in hormone replacement therapy can reveal subjects with reversal phenotype later in life.

1.4.3. Clinical features

CHH is associated with a variable non-reproductive phenotype which includes, for example, the lack of smell (anosmia) or reduced capability to smell (hyposmia), cleft lip or/and palate, renal agenesis, mirror movements of hands (synkinesia), dental defects, micropenis, and cryptorchidism (**Table 1**) (Laitinen *et al.* 2011, Costa-Barbosa *et al.* 2013, Krams *et al.* 1999, Bailleul-Forestier *et al.* 2010, Molsted *et al.* 1997). The presence of these clinical cues can aid in the evaluation of an adolescent with suspected hypogonadism (Costa-Barbosa *et al.* 2013). Additionally, the non-reproductive features emphasize the need for a careful physical examination with special attention to the genitalia, teeth, and the neurological examination, when evaluating a patient with late puberty. Patients with CHH appear to grow relatively normally during childhood, but the impaired pubertal development results in the absence of growth spurt (Raboch & Reisenauer 1976, Kaushanski & Laron 1979)

1.4.4. The differential diagnosis between CHH and constitutional delay of growth and puberty

The early diagnosis of CHH remains as a dilemma (Boehm *et al.* 2015). Distinguishing prepubertal patients with CHH from those with CDGP, especially boys, is the most challenging process (Harrington & Palmert 2012). The only definite method to separate these two groups is to wait for the CDGP patients to show a complete and spontaneous progression of puberty. The difficult differential diagnosis between CDGP and CHH can result in an unnecessary delay in the diagnosis of CHH (Boehm *et al.* 2015). Several clinical and biochemical markers with varying success are used in this diagnostic process (reviewed in Harrington & Palmert 2012, Palmert & Dunkel 2012) (**Table 2**). These include GnRH and hCG stimulation tests, sex hormone levels, and the levels of Sertoli cell markers (**Table 2**). However, none of the tests show a complete diagnostic accuracy (**Table 2**) (Segal *et al.* 2009, Dunkel *et al.* 1985, Coutant *et al.* 2010, Adan *et al.* 2010, Grinspon *et al.* 2010), and most of the studies have been retrospective (Segal *et al.* 2009, Coutant *et al.* 2010, Sequera *et al.* 2002). Additionally, the absence of clinical signs used in the differential diagnostics in these studies is striking. The effectiveness of clinical cues in predicting the progression of puberty, such as cryptorchidism, has not been studied before. Thus, clinicians who evaluate patients with DP would benefit from such clinical predictors of pubertal outcome.

Table 2. The suggested cutoff levels, sensitivities, and specificities of clinical and biochemical markers used to differentiate between congenital hypogonadotropic hypogonadism and constitutional delay of growth and puberty.

Marker(s)	Suggested cutoff levels	Sensitivity (%)	Specificity (%)	Reference(s)
Baseline LH, IU/L	0.3-0.7	85-100	88-90	(Grinspon *et al.* 2010, Binder *et al.* 2015)
Baseline FSH, IU/L	0.9-1.2	88-100	55-88	(Segal *et al.* 2009, Grinspon *et al.* 2010)
GnRH induced LH response, IU/L	2.8-5.8	90-100	84	(Segal *et al.* 2009)
GnRH induced FSH response, IU/L	3.7-4.6	90	52-80	(Grinspon *et al.* 2010, Sequera *et al.* 2002, Binder *et al.* 2015)
Testosterone, nM	0.5	93	100	(Segal *et al.* 2009, Grinspon *et al.* 2010)
Inhibin B, pg/mL	28-35	80-100	75-100	(Segal *et al.* 2009)
hCG test, nM	3.6	92	92	(Coutant *et al.* 2010, Rohayem *et al.* 2015)
AMH, pmol/L	110	80	100	(Coutant *et al.* 2010)
Testicular volume (mL)	3	93	45	(Segal *et al.* 2009)
Combinations				
Inhibin B and LH (pg/mL, IU/L)	35 and 0.5	100	100	(Coutant *et al.* 2010)

Values lower than the cutoff levels increase the probability of CHH

1.4.5. Treatment

The treatment options for CHH depend on the aim of the treatment. Similarly to the treatment of CDGP, sex hormones are used in CHH to induce secondary sexual characteristics and height growth (reviewed in Boehm *et al.* 2015). In infants, cryptorchidism should be corrected during the first year of life as the untreated undescended testes are associated with impaired future fertility potential (Lee *et al.* 1996, van Brakel *et al.* 2013, van Brakel *et al.* 2014). Similarly, short term low-dose testosterone treatment in early infancy induces sufficient penile growth in patients with micropenis (Hatipoglu & Kurtoglu 2013, Landier, Chaussain & Job 1984). The adolescent boys are treated with testosterone and girls with estrogen to induce virilization and pubertal growth. The treatment reduces the risk for psychosocial disturbances caused by sexual infantilism (Dwyer *et al.* 2014). The dosage of sex steroids is gradually increased, mimicking the physiological rise in sex steroid levels that occurs during puberty. Also, the secondary sexual characteristics can be achieved by using pulsatile GnRH or hCG alone or in combination with recombinant FSH (Raivio, Wikstrom & Dunkel 2007, Zacharin *et al.* 2012). These treatment modalities induce testicular growth, spermatogenesis, and are associated with improved HRQoL scores (Boehm *et al.* 2015, Shiraishi, Oka & Matsuyama 2014). However, the previous patient series have included only a limited number of subjects, and the results need to be repeated in a large sample of CHH patients. After the induction of puberty, the

adequate sex hormone dosage is titrated by using the baseline levels of testosterone or estradiol.

1.4.6. Long-term impact of sex steroid deficiency on bone-health and health-related quality of life

Pubertal development is crucial for a normal acquisition of bone mineral content and bone density. Findings in menopausal women have shown a direct negative relationship between estrogen levels and bone density (Ahlborg *et al.* 2003). Similarly, hypogonadal men have a lower bone mineral density than those with normal testosterone levels (Canale *et al.* 2000). Patients with CHH and insufficient hormone replacement therapy have an increased risk for osteoporosis (Laitinen *et al.* 2012a, Finkelstein *et al.* 1989). Hormone replacement therapy restores bone health and emphasizes the life-long need of hormonal replacement therapy in patients with CHH (Laitinen *et al.* 2012a, Canale *et al.* 2000).

Hypogonadism has adverse effects on general well-being and psychosocial long-term outcomes. HRQoL scores in men with CHH suggest that CHH is associated with an impaired quality of life (Aydogan *et al.* 2012, Shiraishi, Oka & Matsuyama 2014, Dwyer *et al.* 2014, Lasaite *et al.* 2013). These studies included mixed study populations and an unclear characterization of CHH (Aydogan *et al.* 2012, Shiraishi, Oka & Matsuyama 2014, Lasaite *et al.* 2013). Aydogan *et al.* reported lower HRQoL scores in men with CHH, particularly in general health and emotional domains of HRQoL (Aydogan *et al.* 2012). These men had higher depression scores when evaluated with Beck Depression Inventory (BDI). After the beginning of testosterone therapy, both the HRQoL and BDI scores improved (Aydogan *et al.* 2012). The studies, however, did not evaluate the impact of the non-reproductive phenotype of CHH or the age of diagnosis on HRQoL.

2. THE HORMONAL REGULATION OF GROWTH

Human growth has been categorized into three different components (infancy, childhood, and puberty) by using the Karlberg's growth model (Karlberg 1989). Each phase is controlled by a distinct hormonal and biological mechanisms: for example, sex steroids are the major regulators of growth during puberty (Karlberg 1989). During the last decade, several studies in both developing and developed countries have reported an increase in mean height (Saari *et al.* 2011, Cole 2000, Roelants, Hauspie & Hoppenbrouwers 2009). This trend is probably due to the changes in environmental factors such as nutrition that regulate growth. Due to this secular trend observed also in Finland, national growth references should be updated from time to time (Saari *et al.* 2011, Sorva *et al.* 1990, Sorva, Tolppanen & Perheentupa 1990).

2.1. Fetal life and infancy

Fetal growth is dependent on materno-placental environment, and the main hormonal contributors to growth are insulin, IGF-1, and IGF-2 (Murray & Clayton 2013). In rodents, the expression of *Igf2* is widespread in fetal tissues during the last trimester, and deleting *Igf1* or *Igf2* results in fetal growth retardation (Fowden 2003). Elevated transcription of *IGF-2* results in overgrowth (Sparago *et al.* 2004), whereas a down-regulated *IGF-2* expression is associated with perinatal growth

retardation (Gicquel *et al.* 2005). Altogether, linear growth in early gestation is more rapid in boys (Pedersen 1982). Growth velocity increases during the first two trimesters and peaks at mid-gestation (2.5 cm/week) (Underwood & Van Wyk 1992). The role of androgens in this process is unclear. Infants with congenital adrenal hyperplasia (CAH), which is a condition of fetal androgen excess, have a higher birth length than healthy controls. Conversely, the newborns with complete androgen insensitivity syndrome (CAIS) show similar birth length to the general population (Balsamo *et al.* 2006, Miles *et al.* 2010). These findings suggest that androgens may contribute to fetal growth.

Very little is known about the contribution of sex steroids on the growth during infancy as well. The transient postnatal activation of the HPG axis appears to interact with an early infancy growth. During minipuberty, serum testosterone levels, but not estradiol, correlates with penile growth and growth velocity (Boas *et al.* 2006, Kiviranta *et al.* 2016). Boys experienced a length velocity up to 4 cm/yr faster than the girls during minipuberty. The peak growth velocity occurs simultaneously with the average age of the peak gonadal activation (at 1 month of age) (Kiviranta *et al.* 2016). Interestingly, the levels of IGF-1 remain similar in boys and girls during the first six month of life, which suggests that the GH/IGF-1 axis is not stimulated despite the elevated sex steroid levels (Kiviranta *et al.* 2016). These findings suggest that androgens modulate growth in early infancy, and the influence appears to be mediated directly on target tissues.

During the first two years of life, infants experience catch-up or catch-down growth which suggests that early infancy growth compensates the growth during fetal life. In detail, birth size correlates poorly with parental size, whereas after the first two years the correlation improves significantly (Murray & Clayton 2013). The phenomenon has been explained with a central mechanism that detects and balances the difference between the expected and the actual size, or that it is a result of compensatory mechanisms of growth plate stem cells to growth delay (Murray & Clayton 2013). The growth during infancy is controlled by the combined effects of thyroid hormone, GH/IGF-1 axis, and nutrition. Diet induces IGF-1 production in the liver (Socha *et al.* 2011, Ong *et al.* 2009, Low *et al.* 2001). Besides liver, IGFs are produced in the target tissues. Locally produced IGF-1 is a more potent growth contributor than the liver produced molecule, since in mice, removing the hepatic *igf1* does not inflict a significant growth retardation (Liu, Yakar & LeRoith 2000). In humans, the phenotype of genetic IGF-1 deficiency includes deafness, intellectual disability, and pre- and postnatal growth retardation (Netchine *et al.* 2011). The most important target tissue of IGFs is the epiphyseal growth plate. In the growth plate, linear bone growth is achieved by the endochondral ossification. Chondrocytes not only express receptors for IGF-1, but also produce IGF-1 locally. In mice, the chondrocyte-derived IGF-1 and IGF-2 contributes significantly to the linear bone growth (Smink *et al.* 2002). Consequently, the infancy growth is first a continuation of the rapid fetal growth. Length velocity is at the highest level during the first 3 months (20 cm/year), and then it gradually declines to 9 cm/yr at the average age of 3 yrs (Thiering *et al.* 2012, Molinari, Gasser & Largo 2013).

2.2. Childhood

After infancy, the primary role of nutritional component in the regulation of growth is replaced by GH stimulation on IGF-1 synthesis in liver and in target tissues

(Murray & Clayton 2013, Chellakooty *et al.* 2006, Rosenfeld 2006, Baumann 2001). At the same time, the importance of thyroid hormone in the growth regulation increases. Hypothyroidism results in the reduction of GH and IGF-1 levels, and also an impaired chondrocyte proliferation and hypertrophy in the growth plate (Schmid *et al.* 2006, Kindblom *et al.* 2001, Stevens *et al.* 2000). The influence of sex steroids on childhood growth is unclear. Supraphysiological levels of androgens, if aromatized to estrogens, promote growth since children with excessive androgen production (*i.e.* CAH) have higher growth velocity and BMI values and a higher risk of childhood obesity than their peers (Volkl *et al.* 2006) (Merke *et al.* 2000). This suggests a programming contribution of androgens to the body composition during childhood. Furthermore, obese children show increased levels of dehydroepiandrosterone, a more advanced bone age throughout childhood, and an increased height standard deviation score (SDS) at the onset of puberty (Denzer *et al.* 2007, Johnson *et al.* 2012, Lass *et al.* 2011). This has been thought to result from an increased aromatase activity in the adipose tissue (Polari *et al.* 2015). In contrast, hypogonadism has been considered to contribute little to childhood growth. Importantly, male patients with androgen insensitivity syndrome have a tendency towards increased height growth during late-childhood and adolescence (Hellmann *et al.* 2012). Children with very rapid infant weight gain have higher levels of adrenal androgens during childhood (Ong *et al.* 2004). An excessive weight gain associates with an earlier pubertal development (Lee *et al.* 2016), albeit a certain amount of weight is critical for the normal onset of puberty (Frisch 1994). All in all, childhood growth is mainly GH dependent, which results in an average annual height velocity of 6 cm in mid-childhood which then declines to 5 cm/yr before the onset of puberty (Kelly *et al.* 2014). Further, the height velocity continues to decline steadily in a case of an absent pubertal growth spurt (Rikken & Wit 1992).

2.3. Puberty

During puberty, the constant childhood growth is gradually changed to the rapid pubertal growth. During the transition, childhood growth slows to its nadir right before the pubertal growth acceleration, which results in a period of very slow height velocity (*i.e.* peripubertal growth dip) (Rogol, Roemmich & Clark 2002). Pubertal growth has been traditionally categorized into three phases: the time of growth acceleration (*i.e.* take-off velocity) right before the actual growth spurt, the time of most rapid growth (*i.e.* peak height velocity), and the phase of decreased growth velocity and the fusion of the epiphyseal cartilage (Tanner *et al.* 1976). The peak pubertal height velocity is reached at 1 to 2 yrs after the onset of puberty (Wehkalampi *et al.* 2011, Abbassi 1998), and girls reach it approximately 2 yrs before boys, which is likely due to the more rapid rise in estradiol levels reported in early pubertal girls (Marshall & Tanner 1970, Janfaza *et al.* 2006). During the growth spurt, the average height velocity is 8 cm/yr in girls, whereas boys exhibit even faster growth (9 cm/yr) (Abbassi 1998). Then the height velocity steadily decreases as the epiphyseal plates fuse. In both sexes, estrogen has a pivotal role in the induction of the growth spurt (Juul 2001, Morishima *et al.* 1995, Carani *et al.* 1997). Estrogen levels stimulate pituitary GH release and IGF-1 production in the liver and in target tissues (Venken *et al.* 2005, Mauras *et al.* 1987). More evidence supporting the role of estrogen in the stimulation of GH/IGF-I axis has accumulated from studies in boys treated with AIs. Adolescent boys treated with AI (anastrozole or letrozole) showed a decrease in IGF-1 concentrations during

follow-up (Mauras *et al.* 2000, Neely *et al.* 2014), whereas one study found no change in IGF-1 levels during anastrozole-treatment (Mauras *et al.* 2008). Treating CPP patients with a GnRH analog decreases not only the levels of gonadotropins and sex steroids but also the levels of IGF-1 and IGF-binding protein 3 (Muller *et al.* 2000, Juul *et al.* 1995). Additionally, estrogen induces growth plate senescence and maintains bone health by stimulating chondrocytes and osteoblast invasion in the epiphyseal plate (Shim 2015, Kusec *et al.* 1998, Yin *et al.* 2015). In boys, estradiol levels correlate positively with bone mineral density (BMD) (Doneray & Orbak 2010). The highest bone mineral content is achieved earlier in girls, whereas boys accumulate higher bone mass and more pronounced bone expansion (*i.e.* periosteal apposition) mainly due to longer period of bone acquisition (reviewed in Bonjour & Chevalley 2014). During puberty, testosterone induces protein synthesis and lipolysis, and increases muscle mass and BMD (Doneray & Orbak 2010, Mauras *et al.* 2003, Hero *et al.* 2006). Thus, adolescent boys accumulate significantly more fat free mass in contrast to girls who gain more fat mass during the same period (Fintini *et al.* 2011, Garnett *et al.* 2004). Altogether, the absence of puberty results in low bone mineral content, osteoporosis, and the absence of pubertal growth spurt which are all observed in patients with CHH (Laitinen *et al.* 2012a, Van Dop *et al.* 1987).

2.4. Adult height

Adult height is regulated by genetic factors. Interestingly, a genome-wide association study found 423 loci, but could not focus single genes, that influenced on adult height (Wood *et al.* 2014). Evidence suggests that the majority of these genes do not participate in the GH/IGF-1 axis, but are associated with for example fibroblast growth factor signaling (Wood *et al.* 2014, Lui *et al.* 2012). After the growth spurt, the adolescents continue to grow until the epiphyseal plates fuse. Thus, the deficiency of sex steroids and the absence of puberty are likely to result in a longer period of growth and a taller adult height. In fact, this is best demonstrated in the patients with aromatase deficiency or with CHH as they typically exceed their genetic growth potential and their adult height is above the height of the general population (Morishima *et al.* 1995, Raboch & Reisenauer 1976, Dickerman, Cohen & Laron 1992, Uriarte *et al.* 1992). Conversely, a premature closure of the epiphyseal plates reduces the adult height. In patients with CPP, a short childhood growth phase together with an early timing of the growth spurt and bone maturation result in a height loss and shorter adult height in contrast to the general population (Carel & Leger 2008, Bertelloni & Mul 2008).

2.5. Evaluation of growth

Adult height estimates are used by clinicians to predict the height reached after puberty. Genetic growth potential can be estimated with the mid-parental target height (MPH). In Finland, the MPH is calculated by using the formula: 0.886 x ([father's height + mother's height] / 2 + 6.8 – 178.9066) / 6.6784 – 0.071 (Saari *et al.* 2011). However, MPH is only a rough estimate of the growth potential regardless of the individual variation in pubertal timing and bone maturation. Statural growth continues until the epiphyseal cartilages fuse. Thus, a more precise estimate of the individual growth potential at a certain calendar age is achieved by determining the current bone age from the wrist and hand x-ray. Bone age is visually estimated by using the radiographic atlases of skeletal maturation

(Tanner *et al.* 1983, Greulich & Pyle 1959). Subsequently, the individual timing of puberty (precocious, normal, and delayed) and the determined bone age are subjected to an adult height prediction model which produces a prediction of adult height (Bayley & Pinneau 1952). As the bone age estimate depends solely on the view of the assessor, it is susceptible to variation. Recently, the discovery of computerized assessment of bone age has made the evaluation of skeletal maturation easier and the adult height predictions more precise (Thodberg *et al.* 2009). This automated bone age calculation is widely implemented to the clinical practice.

Growth velocity may provide cues of chronic illnesses, albeit a number of conditions may manifest in impaired growth (Tuchman *et al.* 2008, Taylor *et al.* 2012, Sankilampi *et al.* 2013). The evidence that support growth velocity to be used in the screening chronic diseases is inadequate. A recent review collected data from 69 articles which reported growth monitoring algorithms to detect chronic growth inhibiting disorders such as GH deficiency, Turner syndrome (TS), celiac disease, and cystic fibrosis (Scherdel *et al.* 2016). The meta-analysis found out that only the clinical decisions rules to detect patients with TS, coeliac disease and cystic fibrosis had sufficient specificities (Scherdel *et al.* 2016). In accordance, the evidence on the diagnostic performance of growth velocity to detect subjects with an abnormal pubertal development is scarce and limited to delayed puberty (Palmert & Dunkel 2012). It has been suggested that the annual growth velocity less than 3 cm could aid in identifying patients with delayed puberty caused by a chronic disease (Palmert & Dunkel 2012). Conversely, a steep growth curve together with advanced bone age in subjects with clinical signs of CPP supports the premature activation of the HPG axis (Fuqua 2013).

AIMS OF THE STUDY

This study was conducted to evaluate the interactions between the HPG axis and growth from early infancy to adult height, and to describe the etiology and outcome of delayed puberty. The specific aims were:

1. to investigate if circulating MKRN3 levels could serve as a read-out of the diminishing central restrain on the HPG axis (I)

2. to characterize the diagnoses that underlie delayed puberty and predictors of its clinical course (II)

3. to depict the growth patterns of patients with congenital hypogonadotropic hypogonadism from birth to adult height (III-IV)

4. to evaluate the health-related quality of life in men with CHH (V)

PATIENTS AND METHODS

3. STUDY SUBJECTS

3.1. Boys with idiopathic short stature (ISS) (I)

The boys with idiopathic short stature (ISS) participated in a randomized placebo-controlled double-blind trial, in which 16 boys were treated with letrozole (Lz) (2.5 mg/d) and 14 received placebo (Pl) for two years (Hero, Norjavaara & Dunkel 2005). At the beginning of the study, the boys had a mean age of 11 years (range: 9.1 - 14.2 yrs). The chronic diseases that can cause short stature were excluded by using growth curve analysis, serum IGF-I level, and in subject with a suspicion of GH deficiency, a GH stimulation test. The boys were investigated every 6 months for 2 years, and the follow-up protocol included a final visit at 36 months after the beginning of the study. At the visits, testis size was calculated by using the formula: length x width^2 x 0.52, and pubertal stage was recorded (Marshall & Tanner 1970, Behre, Nashan & Nieschlag 1989). At the beginning of the study, 28 boys were prepubertal (G1), one boy was at G2 (testicular volume above 3 mL), and one at G3 (Hero, Norjavaara & Dunkel 2005). During the two years of the treatment, eight boys who were treated with Lz and eight who received Pl showed signs of puberty progression, whereas at 36 the months visit seven out of 10 with Lz and six out of 11 boys with Pl were pubertal (Hero, Norjavaara & Dunkel 2005). Additionally, six boys fulfilled the criteria of delayed puberty (*i.e.* G1 at the age of 14 yrs or older). Letrozole which is a potent third-generation AI, effectively inhibited the estrogen biosynthesis as indicated by low serum estradiol levels in the treated boys despite stimulated testosterone secretion (Hero, Norjavaara & Dunkel 2005, Wit, Hero & Nunez 2011).

3.1.1. Collection and analyses of the serum samples

The serum samples of boys with ISS were used (Hero, Norjavaara & Dunkel 2005). The obtained serum was stored at -20 or -80 C until the hormonal analyses were performed. Gonadotropin levels were measured with an ultrasensitive immunofluorometric assays (Wallac, Turku, Finland), inhibin B levels with immunoenzymometric assay (Serotec, Oxford, UK), and testosterone levels with a modified RIA (Hero, Norjavaara & Dunkel 2005). Serum MKRN3 levels were quantified by using the commercially available Human MKRN3 enzyme-linked immunosorbent assay (ELISA) kit (MyBioSource, San Diego, CA, USA) with the sensitivity of 7.8 pg/mL, the within assay CV of 6%, and the between assay CV of 9.2%. Before the analyses, the samples were diluted (1:8) according to the instructions in order to lower the MKRN3 levels to the measuring range of the kit. The final MKRN3 level was achieved by multiplying the measured level with 8.

3.2. Patients with DP (II)

The patients (408 boys and 181 girls) had been investigated for DP at the Pediatric Endocrine Outpatient Clinic of Helsinki University Hospital between 2004 and 2014 and were identified with an ICD-10 code-based inquiry to the electronic patient information system (**Figure 4**). The used ICD-10 codes were E28, E28.3, E28.8, E28.9, E29, E29.0, E29.1, E29.8, E29.9, E30.00, E30.09, E30.8, and E30.9.

Of the 589 patients, 244 (174 boys and 70 girls) fulfilled the criteria of DP (**Figure 4**). The medical records included information about Tanner stages in 244 (100%), bone age in 227 (93%), baseline LH 216 (88%) and FSH levels in 216 (88%) and 218 (89%), sex steroid levels in 217 (89%). Of the second line investigations of DP, a GnRH test was performed in 104 (43%) and inhibin B levels in 126 (72%) boys. A growth hormone (GH) stimulation test was performed in 15 patients (8 boys and 7 girls), and two of them (panhypopituitarism and juvenile rheumatoid arthritis) had a result which suggested a GH deficiency. A ruler-measured testicular volume was available in 120 (69%) subjects. Height measurements, which were taken at least every six months, were available in 230 (94%) boys, which enabled the calculation of the annual height velocity. The timing of puberty in the first degree relatives of the patients was recorded in all patients, and puberty was considered late if a sister or the mother had reached menarche after 14 yrs, or a brother or the father had experienced late puberty or delayed growth spurt.

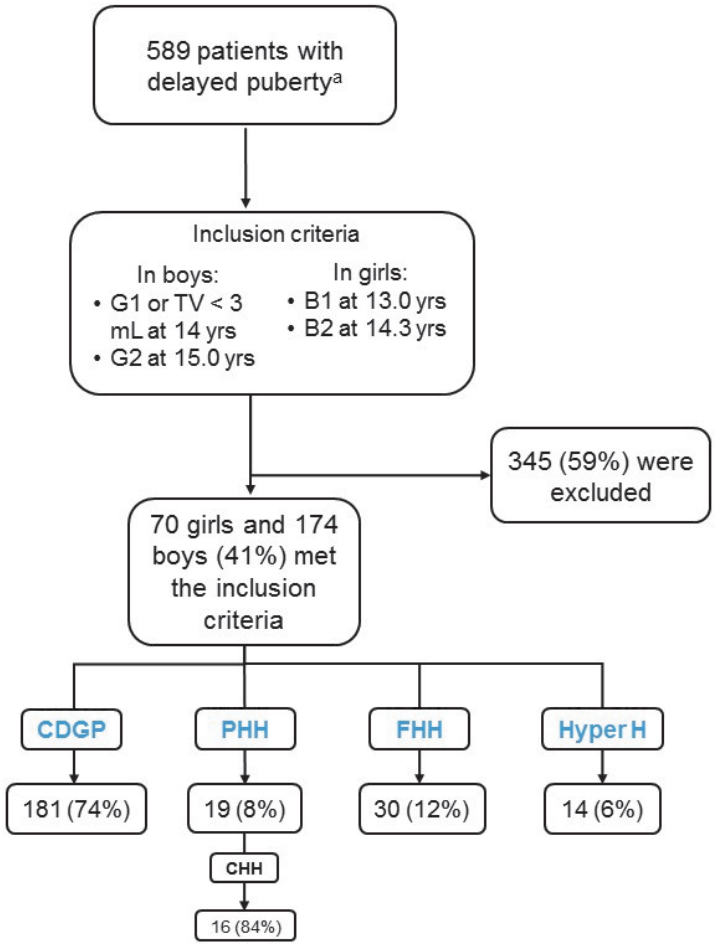

Figure 4. A flowchart describing the patient recruitment for study II. [a] Patients were identified by an ICD-10 code-based inquiry (E28, E28.3, E28.8, E28.9, E29, E29.0, E29.1, E29.8, E29.9, E30.00, E30.09, E30.8, and E30.9) (Modified from II).

3.2.1. Classification of different DP types

The diagnoses of DP were classified into the four main diagnostic categories: FHH, PHH, Hyper H, and CDGP (Sedlmeyer & Palmert 2002). The diagnosis of CDGP was confirmed when the clinical, the hormonal, and the radiologic data ruled out pathological causes of DP. Further, in most of the cases with CDGP (86%), the progression of puberty was confirmed in the hospital outpatient clinic, whereas the remaining cases were followed up in the school health care. However, none of the patients were referred back to the tertiary center for an evaluation of pubertal progress.

The PHH group consisted of patients with a confirmed pathology in the CNS, or an organic cause for the hypogonadism, and/or low LH and FSH and sex steroid levels, and without any spontaneous progression of puberty until the age of 18 yrs. Of the 19 patients with PHH, 16 (11 boys and five girls) had CHH as the cause of the permanent hypogonadism. In the remaining three patients, PHH was caused by Prader-Willi syndrome, panhypopituitarism, and craniopharyngioma.

The patients with FHH had an identified disease that likely delayed pubertal onset, but the patients showed a spontaneous progression of puberty during the follow-up. Interestingly, a spontaneous progression of puberty was confirmed in 14 girls and 15 boys (97%) with FHH within the next three yrs after the initial evaluation of puberty. The patients with FHH had no hormonal or clinical signs which suggested the presence of permanent hypogonadism. Additionally, the FHH group included patients with anorexia nervosa and those with severe underweight. These patients had age- and sex-matched BMI (ISO-BMI) values below 16 kg/m^2 prior to the initial evaluation (Saari *et al.* 2011). Finally, the diagnosis of Hyper H was confirmed with gonadal failure and high baseline LH and FSH levels. Remarkably, a history of prior cryptorchidism was reported in the three boys with CDGP (unilateral) and in four boys with CHH (two unilateral and two bilateral), whereas none of the patients had a history of micropenis. The methods used to determine the levels of gonadotropins, sex steroids and inhibin B between 2004 and 2014 are described in Table 3 (**Table 3**).

Table 3. The methods used to determine gonadotropins, sex steroids and inhibin B levels in studies II-V.

Hormone(s)	Method	Period	Detection limit	Manufacturer	Study
LH and FSH	Immunofluorometric assay	2004 - 2011	0.1 IU/L	AutoDELFIA, Wallac, Turku, Finland	II, III, IV, V
	Electrochemiluminescence immunoassay	2011 -	0.1 IU/L	Roche Diagnostics, USA	II
Estradiol	Radioimmunoassay	2004 - 2009	30 pmol/L	DiaSorin S.p.A,Vercelli, Italy	II
	Radioimmunoassay	2009 - 2014	30 pmol/L	AutoDELFIA, Perkin-Elmer, Turku, Finland	II, IV
	Immunoluminometric assay or mass spectrometry	2014 -	10 pmol/L	Siemens Healthcare Diagnostics, Germany	II
Testosterone	Radioimmunoassay	2004 - 2005	< 1.0 nmol/L	Packard-Becker, Groningen, The Netherlands	II
	Mass spectrometry	2005 -	0.05 nmol/L	AB Sciex, Foster City, California, USA	II, III, V
Inhibin B	ELISA	2004 - 2010	7 pg/mL	Oxford Brooks Innovation and Diagnostics Systems Laboratories, UK	II
	ELISA	2010 -	2.6 pg/mL	Beckman Coulter, Inc. USA	II

3.3. Patients with CHH (III-V)

The third study cohort included 36 patients (30 men and 6 females) with CHH who participated in the nationwide study of KS in Finland (Laitinen *et al.* 2011). In addition to the Finnish boys with CHH, the growth data of nine Danish patients were included (Tommiska *et al.* 2014). The diagnosis of CHH was based on i) absent or incomplete puberty at the age of 18 years and ii) low sex steroid levels accompanied by normal or subnormal gonadotropin levels and iii) otherwise normal anterior pituitary function and iv) no organic cause for their condition. Seven boys (six Finns and one Dane) did not meet the criteria since they were less than 16 yrs old at the time of diagnosis. Their diagnosis, however, was confirmed since the Finns had a history of prior cryptorchidism and/or micropenis, and no testicular growth during the clinical follow-up, whereas the Dane had a micropenis and very low minipubertal LH (< 0.05 IU/L) and testosterone (< 0.23 nmol/L) levels. The molecular genetic diagnosis of CHH was confirmed in 22 patients (Laitinen *et al.* 2011, Laitinen *et al.* 2012b, Tommiska *et al.* 2014). The girls all had a mutation in *FGFR1* (n=6), whereas in boys with CHH, the gene mutations were categorized into three groups: the boys with mutations in *ANOS1* (n=7), *FGFR1* (n=5), and into those with mutations in *gonadotropin-releasing hormone receptor* (*GNRHR*), *chromodomain helicase DNA-binding protein 7* (*CHD7*), or *prokineticin 2* (*PROK2*) (n=4). Clinical signs of profound GnRH deficiency (history of micropenis or/and cryptorchidism) were reported in 23 (53%) (14 Finns and 9 Danes) subjects, whereas one girl had a cleft lip and none had bony anomalies. Hormonal markers were determined in the laboratory of Helsinki University Central Hospital (HUCH). The used methods are described in detail in Table 3 (**Table 3**).

3.3.1. Acquisition of growth data

The growth data of six CHH girls with an *FGFR1* mutation, and 36 boys with CHH (27 from Finland and 9 from Denmark) were reviewed retrospectively (Laitinen *et al.* 2011, Laitinen *et al.* 2012b, Tommiska *et al.* 2014). Height/length and weight were measured by trained nurses, and bone age was determined by the physician according to the Greulich and Pyle atlas. National reference data were used to calculate the height/length SDSs. We were especially interested in the linear growth during minipuberty. Thus, growth charts were carefully searched to obtain the length measurements at birth, and three and six months of age. When an exact measurement was not present at three or six months of age, we selected the nearest available measurement (at 3 months, range: 2-4 months, and at 6 months, range: 5-7 months). Fifteen (42%) of the boys with CHH had representative length measurements (*i.e*. birth length and a length measurement at three or six months of age) during minipuberty, which allowed us to calculate the decline in length SDS from birth to three and six months of age. Subsequently, the length SDSs were compared with MPH SDSs, available in 11 (73%) boys with representative length measurements during minipuberty. Adult heights were available in 31 boys (23 Finns and 8 Danes) and in three girls. The MPH SDS (n=22) was calculated in Danes with the mean height SDS of mother and father, and in Finnish boys and girls by using the equations: 0.886 x ([father's height + mother's height] / 2 + 6.8 – 178.9066) / 6.6784 – 0.071, and 0.0611 x (father's height + 0.0703 x mother's height - 22.37), respectively (Saari *et al.* 2011).

In the boys with CHH, the mean age of pubertal induction was 16.1 yrs (range: 12–21), whereas the corresponding age in girls was 15.7 yrs (range: 14.8–16.8). In 14 boys, the data permitted us to calculate the dosage of testosterone used during the first year of pubertal induction (mg/kg/yr).

Before the age of 2 yrs, weight gain was characterized by using the weight-for-length which is the percentage deviation of weight from the median weight for length and sex (%DW) (Sorva, Tolppanen & Perheentupa 1990). Thereafter, ISO-BMI or BMI was used (Saari *et al.* 2011). The ISO-BMI values were categorized into severe underweight (ISO-BMI less than 16 kg/m^2), underweight (16-17 kg/m^2), normal weight (17-25 kg/m^2), overweight (25-30 kg/m^2), and obesity (more than 30 kg/m^2) (Saari *et al.* 2011).

3.3.2. Health-related quality of life

Health-related quality of life was evaluated in 30 men with CHH by using the 15D instrument (Sintonen 2001). At the evaluation visit, the mean age of the patients was 38.1 yrs (range: 16–61), and four patients had a reversal phenotype of CHH. The 15D is a descriptive questionnaire which includes questions about the 15 different dimensions of health (mobility, vision, hearing, breathing, sleeping, eating, speech, excretion, usual activities, mental function, discomfort and symptoms, depression, distress, vitality, and sexual activity). The answer to each question is classified into 5 levels (the best level=1; the worst level=5), and the alternative that best describes the current state is chosen. Thus, the questionnaire produces a single index score (15D score, scale from 0-1, 1=full health, 0=being dead) which represents the overall HRQoL, and a similar score for each dimension. Subsequently, population-based preference weights are implemented to the 15D scores which result in final comparable HRQoL scores (range 0–1). In this study, we compared the mean 15D score and the mean dimension values in patients with CHH to those of a general male population (n=1227). A clinically relevant difference in the 15D score is 0.03 (Alanne *et al.* 2015).

4. STATISTICS

Statistical analyses were conducted with SPSS statistical software, release 22.0 (SPSS, Chicago, IL, USA). The data is presented with mean (SD) unless otherwise mentioned. The analyses between two groups were carried out with the t-test (I-II, V) or the Mann-Whitney U-test (I-II), whereas when comparing the height SDS to general population, one-sample t-test was used. Longitudinal length/height and weight measurements were analysed with the paired samples t-test. Correlations were determined with Spearman's rank correlation (r_s) (I-III, V). The correlations were two-sided, except for the correlation between the age of diagnosis and HRQoL (V). However, this was accepted since delayed diagnosis has been shown to associate with long-term psychological consequences (Dwyer *et al.* 2014).

Categorical variables were analysed with the Fisher's exact test. Odds ratios (OR) with 95% confidence intervals (CI) were calculated (II). The diagnostic accuracy of the clinical and hormonal markers of puberty were modelled with the receiver operating characteristic (ROC) curves with area under the ROC curve (AUC) and 95% CI. The ROC curves were interpreted and cutoff values which

maximized the sensitivity and the specificity were selected. For the risk prediction model of CHH in study II, the quality control (QC) measures of inhibin B were obtained between 2004 and 2014, and fitted in to a linear model that predicted the dispersion for each mean. Based on mean ± 2 standard error of the QC measurements, three inhibin B categories were created (10-49, 49-111, and 111-212 ng/L). Subsequently, inhibin B levels of the patients were divided into the three categories. Finally, the combination of inhibin B category and testis size was subjected to a logistic regression model which predicted the disease risk.

The longitudinal measurements of MKRN3, gonadotropins, testosterone, and inhibin B were investigated by implementing the method of summary measures (Matthews *et al.* 1990). Thus, a regression line was fitted to each patient's measurements, with age as an independent variable and circulating MKRN3, testosterone, LH, FSH, or inhibin B levels as dependent variables. The obtained regression coefficients were analyzed as raw data with the t-test. The statistically significant level was set to *P* value less than 0.05.

5. ETHICS

The study protocol was approved by the Ethics Committee of the HUCH and Danish regional ethical committee, and the national agency of medicines (FIMEA) (I, III-V). A written informed consent was obtained from all subjects and their guardians. The study on the etiology of delayed puberty contained only data from the medical records without any contact to the patients. Thus, according to the Finnish medical research act, no ethical permission was required (II).

RESULTS

1. Serum MKRN3 levels in boys before the clinical onset of puberty

During the three year follow-up, circulating MKRN3 declined significantly (-669 ± 713 pg/mL/yr, *P* < 0.001) (**Figure 5**). The overall decrease was similar between the Lz-treated boys (n=16) and those who received Pl (n=14) (-657 ± 750 *vs* -683 ± 690 pg/mL/yr, *P* = NS). Subsequently, we investigated how MKRN3 levels changed during the progression of puberty, and analyzed the longitudinal changes in MKRN3 levels in 13 boys with available measurements before and after the clinical onset of puberty (defined as genital stage 2). In these boys, the decline in MKRN3 level was more profound before G2 (2931 ± 2750 pg/mL/yr), than after the onset of puberty (560 ± 1510 pg/mL/yr, *P* < 0.05). Interestingly, before pubertal onset, the boys who were treated with Lz had a slower decline in MKRN3 levels (-782 ± 3190 pg/mL/yr) than those who received Pl (-2030 ± 1821 pg/mL/yr, *P* < 0.05).

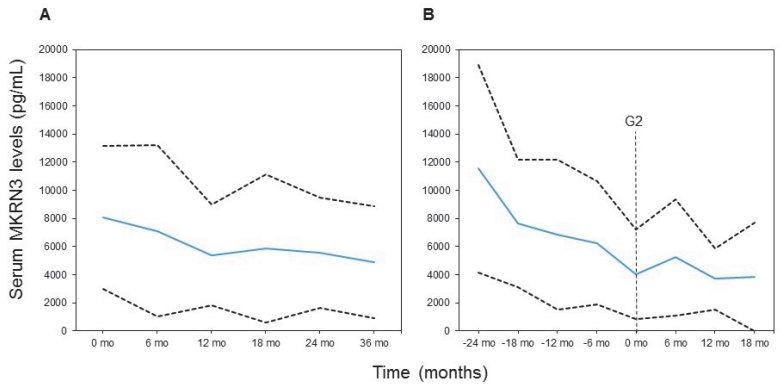

Figure 5. *Panel A*, the mean MKRN3 levels in boys with idiopathic short stature during the follow-up. *Panel B*, the mean MKRN3 levels adjusted for the clinical onset of puberty (genital stage 2, G2). Lines represent mean (blue) ±1 standard deviation (dashed line).

Since *MKRN3* is suggested to be a key inhibitor of GnRH neurons, we examined if circulating MKRN3 levels would associate with the clinical and the biochemical markers of puberty. Indeed, the decline in MKRN3 levels correlated negatively with testis sizes (r_s= -0.3, *P* < 0.001), and the decline appeared to be faster at small testis sizes (**Figure 6**). Additionally, we examined the relationship between testicular growth and MKRN3 by determining the testicular volume for each patient at the time of the highest and the lowest measured MKRN3 level. At the highest MKRN3 level, the mean testicular volume was small (2.0 ± 2.9 mL), whereas larger testes sizes were observed at the lowest MKRN3 level (5.8 ± 4.5 mL, *P* < 0.001). After excluding the two boys who showed an increasing longitudinal change in MKRN3 levels during the study period, we found that the rate of decline in MKRN3 levels (n=26) correlated negatively with the corresponding change in testosterone (r_s= -0.6, *P* < 0.01), LH (r_s= -0.5, *P* < 0.01,

n=26), and inhibin B levels (r_s= -0.44, P < 0.05). The reciprocal association between serum MKRN3 levels and the levels of inhibin B in four individuals are described in Figure 8 (**Figure 7**).

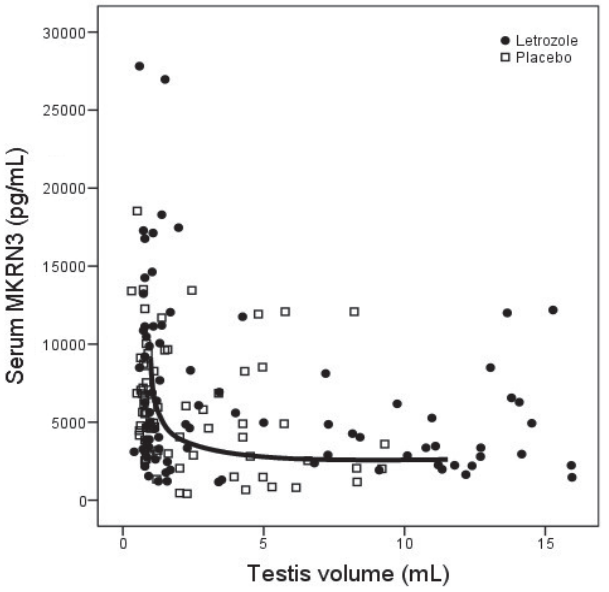

Figure 6. The association between testis size and serum MKRN3 levels (160 measurements) in 30 boys with idiopathic short stature (ISS) followed for 3 yrs (r_s= -0.3, P < 0.001). The line represents the relationship between testis size and MKRN3 in 14 boys with ISS who received placebo (r_s= -0.4, P < 0.001) (Modified from I).

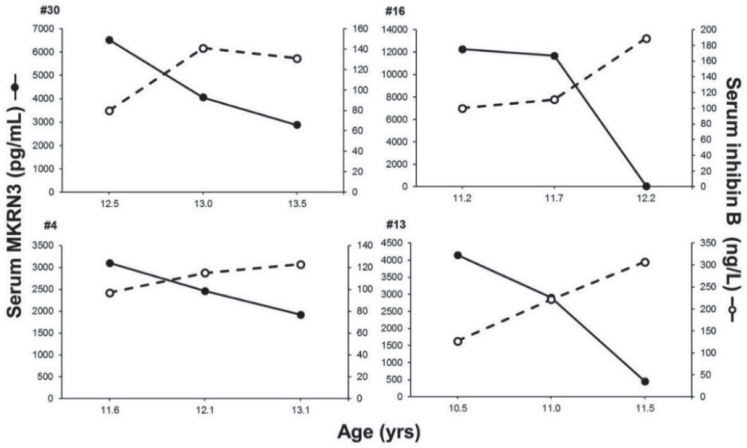

Figure 7. The reciprocal changes in circulating MKRN3 and inhibin B levels in four boys with ISS before and after the onset of puberty.

Prepubertal boys treated with Lz and those with Pl had similar levels of testosterone, gonadotropin and inhibin B levels, testis sizes, and no difference in

the age of puberty onset (Hero, Norjavaara & Dunkel 2005). The six boys who were diagnosed with DP, however, experienced a significantly lower decline in MKRN3 levels than those with normal pubertal timing (-81± 612 *vs* -812 ± 671 pg/mL/yr, $P < 0.05$), albeit we found no association between circulating MKRN3 and parameters of pubertal timing: age at G2, time from the initial evaluation to G2, or bone age at G2 (r_s= 0.05 – 0.3, P = NS).

2. The underlying causes of DP

We found 30 different diagnoses that underlie DP (**Table 4**). In both sexes, the most common cause was CDGP, and it was more frequent in the boys than in the girls (82% *vs* 56%, $P < 0.001$). Additionally, 63% of the boys and 69% of the girls with CDGP had a positive family history of DP (P = NS). The absolute number of girls with familial CDGP (n=27) was lower than the number of boys (n=90). In consequence of the lower number of girls with CDGP, the relative frequency of pathological conditions that underlie DP (*i.e*. FHH, PHH or Hyper H) was higher in girls (44% *vs* 18%, $P < 0.05$). The second most frequent cause of DP in both sexes was FHH. Further, a difference between the sexes was apparent in the FHH and Hyper H categories, since they both affected the girls more frequently than the boys ($P < 0.001$).

Table 4. The number of different diagnoses that underlie delayed puberty at the pediatric outpatient clinic of Helsinki University Central Hospital between 2004 and 2014 (n=244) (Modified from II).

Diagnoses	Male	Female
CDGP	**142 (82%)**	**39 (56%)**
PHH	**13 (7%)**	**6 (9%)**
Congenital hypogonadotropic hypogonadism	11	5
Hypopituitarism	1	
Craniopharyngioma		1
Prader-Willi syndrome	1	
FHH	**16 (9%)**	**14 (20%)**
Severe underweight	4	3
Anorexia	1	2
Severe developmental delay	1	2
Crohn´s disease	2	
Clinical CHARGE syndrome without CHD7 mutation	1	1
VATER assosiation	1	
Cornelia de Lange syndrome		1
Cartillage-hair hypoplasia		1
Celiac disease		1
Intense excercise		1
Jacobsen syndrome and hypothyroidism		1
Hydrocephalus	1	
Pulmonary fibrosis	1	
Juvenile idiopathic arthritis	1	
Systemic lupus erythematosus		1
Radiation therapy for malignancy	1	
CNS disorders (Small Rathke´s cleft cyst or empty cella)	2	
Hyper H	**3 (2%)**	**11 (15%)**
Anorchia	1	
Idiopathic testicular failure	1	
Idiopathic ovarian failure		6
Chemotherapy for malignancy (Askin tumor)		1
Turner syndrome		3
FSH receptor gene mutation		1
SRY-positive XX male	1	

CDGP, constitutional delay of growth and puberty; PHH, permanent hypogonadotropic hypogonadism; FHH, functional hypogonadotropic hypogonadism; Hyper H, hypergonadotropic hypogonadism; CHD7, chromodomain helicase binding protein 7.

Subsequently, we compared the distribution of the four diagnostic categories in our series to the frequencies reported in the most comprehensive study published so far (Sedlmeyer & Palmert 2002). In our series, the frequency of CDGP was significantly higher (in girls 56% and in boys 82%) than reported before (32% and 64%, $P < 0.001$, respectively) (**Figure 8**) (Sedlmeyer & Palmert 2002). By contrast, the frequency of FHH (9%) and Hyper H (2%) in boys, and PHH (15%) in girls, was significantly lower than proportion in the previous series (20%, 7%, and 25%, $P < 0.05$, respectively) (**Figure 8**).

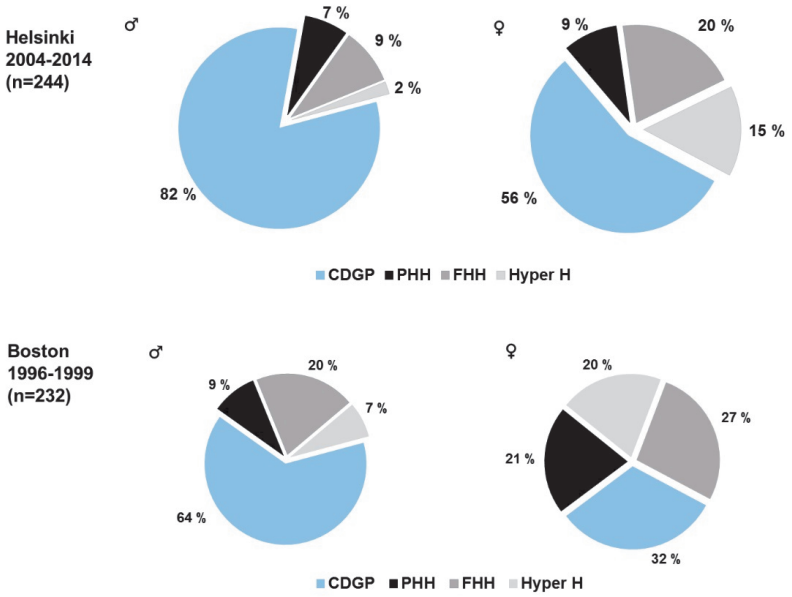

Figure 8. The distributions of the four main diagnostic groups that underlie late puberty in the two largest tertiary center patient series (Study II and Sedlmeyer & Palmert 2002). CDGP, constitutional delayed of growth and puberty; PHH, permanent hypogonadotropic hypogonadism; FHH, functional hypogonadotropic hypogonadism; Hyper H, hypergonadotropic hypogonadism.

3. The differential diagnosis of DP

A history of prior cryptorchidism was more frequent in those with PHH (30%) than in those with CDGP (2%) ($P < 0.05$). Similarly, patients with CHH (a subgroup of PHH) had undescended testes (36%) more commonly than those with CDGP (2%, $P < 0.05$). Thus, OR for a boy with DP and a history of cryptorchidism to have CHH was 26.5 (95% CI; 4.9-142, $P < 0.001$). By contrast, in late maturing boys without a history of cryptorchidism the risk of CHH was low (OR 0.04, 95% CI; 0.01-0.2, $P < 0.001$), and the probability of CHH decreased even more if the boy had also a positive family history of DP (OR 0.01, 95% CI; 0.001-0.1, $P < 0.01$).

Testicular volume, measured with a ruler, inhibin B and GnRH-induced LH levels were the most effective markers to discriminate between the prepubertal (genital stage 1 or testis size < 3mL) boys with CHH (n=10) and those with CDGP

(n=76), whereas equally effective markers could not be found in girls. Additionally, when testis size was combined with inhibin B or GnRH-induced LH levels, the diagnostic efficacy was improved even more. The performance of clinical and biochemical predictors of DP, and their combinations, are described in Table 5 (**Table 5**) and Table 6 (**Table 6**).

Table 5. The clinical and biochemical markers to discriminate between prepubertal boys with congenital hypogonadotropic hypogonadism (n=10) and those with constitutional delay of growth and puberty (n=76).

Predictor	AUC (95% CI)	Cutoff level	Sensitivity (%)	Specificity (%)
Baseline LH, IU/L	0.86 (0.73-0.98)	0.5	100	68
Baseline FSH, IU/L	0.89 (0.75-1.00)	1.2	86	88
GnRH-induced LH, IU/L	0.92 (0.83-1.00)	4.3	100	75
GnRH-induced FSH, IU/L	0.81 (0.6-1.00)	3.3	86	72
Testosterone, nM	0.74 (0.59-0.88)	0.6	91	42
Inhibin B, ng/L	0.88 (0.74-1.00)	60	90	83
Testicular volume, mL	0.95 (0.87-1.00)	1.1	100	91
Combinations				
Inhibin B and testicular volume	0.95 (0.87-1.00)			
GnRH-induced LH and testicular volume	0.98 (0.94-1.00)			

AUC, area under the curve; CI, confidence interval. Values lower level than the cutoff levels increase the probability of CHH.

Table 6. The biochemical markers used to discriminate between prepubertal girls with congenital hypogonadotropic hypogonadism (n=5) and those with constitutional delay of growth and puberty (n=36).

Predictor	AUC (95% CI)	Cutoff level	Sensitivity (%)	Specificity (%)
Baseline LH, IU/L	0.53 (0.26-0.80)	0.3	80	40
Baseline FSH, IU/L	0.82 (0.53-1.00)	1.2	80	95
GnRH-induced LH, IU/L	0.78 (0.43-1.00)	1.7	80	95
GnRH-induced FSH, IU/L	0.80 (0.45-1.00)	4.2	80	95
Estradiol, nM	0.58 (0.34-0.80)	0.05	80	45

AUC, area under the curve; CI, confidence interval. * Estradiol level 0.05 nmol/l is the detection limit of the assay. Values lower level than the cutoff levels increase the probability of CHH.

While in a search for new methods that would aid in the challenging differential diagnosis between CHH and CDGP, we developed a clinical risk prediction model for CHH which takes advantage of the performance of different inhibin B analyte concentrations and testicular volume. In brief, testicular volumes and inhibin B levels were combined in a logistic regression analysis which estimated the probability of CHH in a given inhibin B level and testicular volume (**Table 7**). The

risk for CHH was the highest at small testicular volumes (< 1mL) and low inhibin B levels (< 50 ng/L).

Table 7. The mean probability of CHH according to testicular volume and inhibin B level. The inhibin B categories were calculated from the quality control measurements of inhibin B (mean ± 2 standard deviation) between 2004 and 2014.

Inhibin B (ng/L)	Testis size	
	<1.0 mL	1.1-2 mL
10-49	90 (50-100)*	20 (0-80)
49-111	60 (10-100)	10 (0-40)
111-212	20 (0-90)	0 (0-10)

*Probability is presented with mean percentage (range)

Subsequently, the performance of the growth velocity to differentiate between the four diagnostic categories of DP was evaluated. We found that boys with FHH (n=18) had a lower mean height velocity than those with CDGP or CHH (n=142) (3.2 ± 1.2 *vs* 4.1 ± 1.7 cm/yr, $P < 0.05$, respectively). Since it has been suggested that height velocity below 3 cm/yr could identify patients with FHH from those with CDGP or CHH (Palmert & Dunkel 2012), we categorized the boys and the girls into those whose height velocity was above 3 cm/yr and into those who grew slowly (< 3 cm/yr). In girls, the frequencies of the four diagnostic categories (*i.e.* CDGP, PHH, FHH, and Hyper H) did not differ between the two growth categories. Conversely, in boys, the number of cases with FHH was significantly higher in boys who grew slowly than in those with height velocity more than 3 cm/yr (19% *vs* 4%, $P < 0.05$). However, the ROC analysis of growth velocity revealed that the previously suggested growth velocity cutoff level of 3 cm/yr discriminated the boys with FHH from those with CDGP or CHH only with a sensitivity of 50% and a specificity of 80% (Palmert and Dunkel, 2012). Next we evaluated the efficacy of annual growth velocity to identify the patients with an abnormal brain MRI scan. Brain MRI was performed in 39 patients (24 boys and 15 girls), and it was considered abnormal in 6 cases: one girl with a craniopharyngioma, two boys with a Rathke's cleft cyst, two with a hypothalamic hamartoma, and one with empty cella. The girl with the craniopharyngioma grew very slowly (2 cm/yr), while the mean growth velocity of the boys with an abnormal MRI scan was similar to those with a normal MRI scan (n=18) (3.5±0.9 *vs* 4.2±2.4 cm/yr, P = NS).

4. The growth of patients with CHH from birth to adult height

The boys with CHH (III) showed a significant decrease in the mean length SDS from birth (0.2 ± 1.6 SDS) to 3 months (-0.9 ± 1.2 SDS, $P < 0.01$) and to 6 months of age (-0.7 ± 1.3 SDS, $P < 0.05$). The mean length SDSs at 3 and 6 months of age were lower than their MPH SDSs (-0.02 ± 0.9 SDS, $P < 0.05$) and, at 3 months of age, lower than the length SDSs of the general population (-0.9 ± 1.2 SDS, $P < 0.05$). The presence of minipubertal growth deflection was further evaluated by excluding four patients with birth length above the normal range (±2SD). Despite excluding the four patients, the length deflected from birth (-0.3 ± 1.3) to 3 months

of age (-1.0 ± 1.3, $P < 0.01$). The boys with CHH experienced a reduction in weight-for-length from birth (2 ± 10%) to 6 months of age (-4 ± 10%, $P < 0.05$).

Subsequently, we evaluated separately the growth rate of patients with signs of a severe GnRH deficiency (*i.e.* a history of cryptorchidism and/or micropenis). These males (n=8) experienced a profound length SDS deflection from birth (0.8 ± 1.8 SDS) to 3 (-1.0 ± 1.4 SDS, $P < 0.01$) and to 6 months of age (-0.5 ± 1.2 SDS, $P < 0.05$) (**Figure 9**).

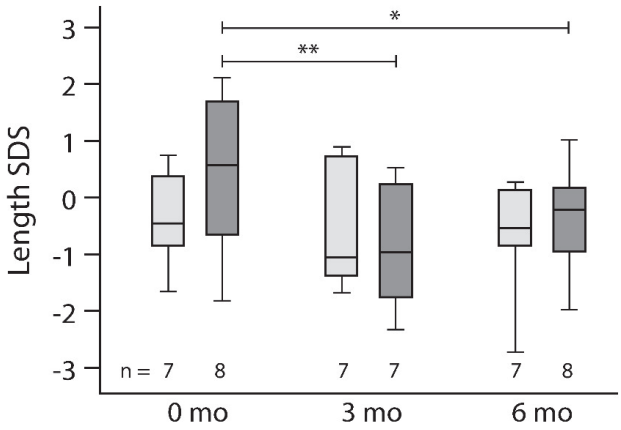

Figure 9. The length standard deviation scores (SDS) of CHH patients with (dark grey) and without (light grey) a history of micropenis and/or cryptorchidism.* $P < 0.05$, ** $P < 0.01$.

The length SDS of CHH patients with verified gene mutations decreased significantly from birth (0.1 ± 1.1 SDS) to 3 months of age (-1.0 ± 0.9 SDS, $P < 0.01$). At 3 months of age, the boys had lower height SDS than the general population (-1.0 ± 0.9 SDS, $P < 0.05$), and the boys with *ANOS1* (n=7) had a lower birth length than those with *FGFR1* (n=4) (-0.7 ± 0.8 *vs* 0.8 ± 0.8 SDS, $P < 0.05$, respectively).

The boys with CHH grew relatively normally during childhood, but at the average age of 7 years (n=31), their mean height SDS (-0.8 ± 1.3 SDS) was lower than their mean MPH SDS (0.07 ± 0.7 SDS, P < 0.05) and the height SDSs of the general population ($P < 0.01$). During adolescence, the height SDS reached its nadir (-1.8 ± 1.4 SDS) at a mean age of 15.8 yrs, and thereafter the boys experienced an increased growth velocity as a result of the induction of puberty. The dosage of testosterone used during the first year of puberty induction (mean dosage 30, range 5 – 114 mg/kg/yr) in 14 patients correlated negatively with the adult height (r_s= -0.7, $P < 0.05$) (**Figure 10A**), but no association was found between the age at puberty induction and the adult height (r_s= 0.1, P = NS). Further, the boys with CHH had a mean adult height of -0.4 ± 1.3 SDS which was similar to the adult heights of the general population, their MPH SDSs, and did not differ between gene mutations (P = NS). We found a decreasing trend in the age at induction of puberty from the 1970s onwards (r_s= -0.4, $P < 0.05$) (**Figure 10B**).

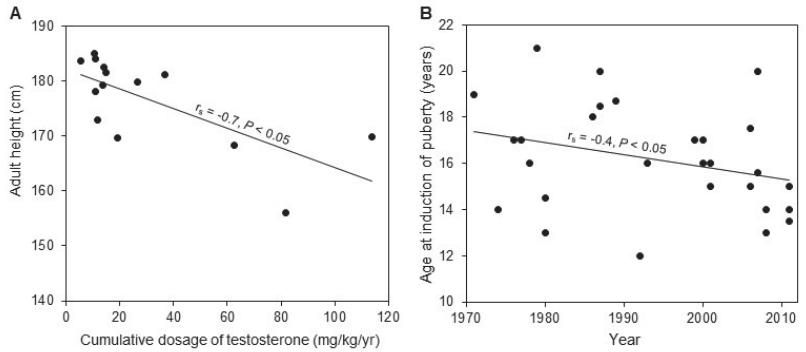

Figure 10. *Panel A*, The correlation between adult height and the cumulative dosage of testosterone during the first year of puberty induction. *Panel B*, the trend in the age of puberty induction between 1970 and 2012 (III).

The girls with CHH (IV) and *FGFR1* mutations experience an unremarkable growth during early infancy, since length SDS did not deviate with the exception of one girl whose height SDS decreased from birth (-0.8 SDS) to 6 months of age (-1.5 SDS). Thus, the transient height SDS deflection seen in boys with CHH boys was not evident in the growth curves of the girls with CHH. Similar to the boys with CHH, the height SDSs of the girls decreased during childhood. The deflection in the height curve began at the age of 4 to 7 yrs, and the mean height SDS of the girls reached its nadir (-1.8 SDS) at the average age of 14.5 yrs, which occurred, as in boys with CHH, right before the induction of puberty.

The late sexual maturation was also apparent in the bone maturation of the girls, since they had a mean delay of 2.4 yrs (range: -3.2–(-1.9) yrs) in the bone age. In the three girls, with available adult heights, the adult height SDSs minus MPH SDSs ranged from -0.2 to 1.2 SDS. All subjects had normal weight development, as their BMI trajectories were within the normal limits.

5. Health-related quality of life in males with CHH

The male patients with CHH had a lower mean 15D score than the age-matched general male population (0.918 ± 0.074 *vs* 0.948 ± 0.067, $P < 0.05$). In particular, the dimensions most affected were depression and distress (**Figure 11**). Although a continuous sex steroid treatment is essential to prevent the long-term consequences of CHH, we found no association between the testosterone levels, or the total duration of hormone replacement therapy pauses and the 15D scores (P = NS). Subjects with a reversal phenotype (n=4) experienced HRQoL similar to the general population, whereas the males with a history of prior cryptorchidism and/or micropenis (n=14) reported significantly lower 15D scores in the dimension of sexual activity ($P < 0.01$). Interestingly, the age at diagnosis correlated negatively with the 15D score (r_s= -0.4, $P < 0.05$) and the dimension of depression (r_s= -0.3, $P < 0.05$).

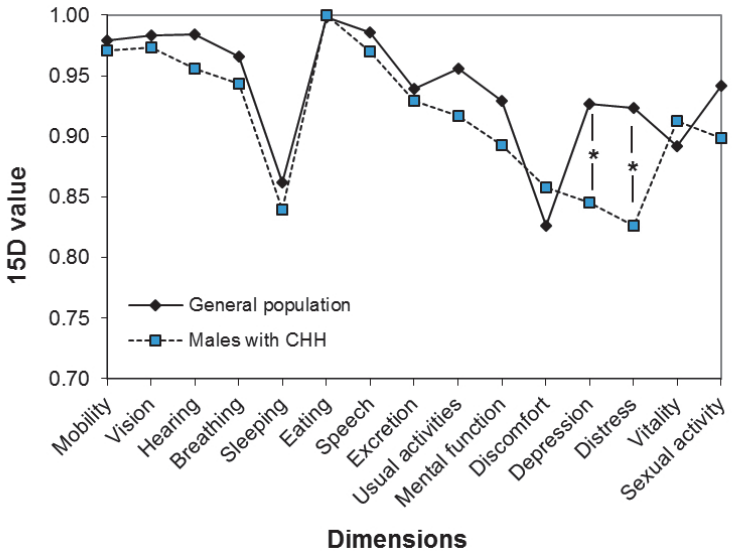

Figure 11. Health-related quality of life assessed with the 15D instrument in males with CHH. A lower 15D value indicates a more severely affected dimension. * $P < 0.05$ (Modified from V).

Eight patients with CHH had been diagnosed with depression, bipolar disease or anxiety disorder, and all of them were on antidepressants or anxiety medication. The subjects who received antidepressants or anxiety medication had lower 15D score and the scores in the dimensions of depression, distress, and usual activities than those without medication ($P < 0.05$). Co-morbidities appeared not to influence on HRQoL, since the patients with or without co-morbidities reported similar 15D scores (P = NS).

The main results of the five studies (I-V) are summarized in Table 8 (**Table 8**).

Table 8. Summary of materials, methods, and main results of the studies.

Study	Material	Methods used	Main results
I	30 peripubertal boys with ISS. 16 were treated with Lz, and 14 received Pl	Serum MKRN3 ELISA, testosterone, inhibin B, gonadotropins, and testicular volume	MKRN3 levels declined during prepuberty, and the decline was slower in boys who were treated with Lz suggesting that estrogen influences on the loosening of the central restrain on the HPG axis. The rate of decline in MKRN3 levels associated inversely with the clinical and biochemical markers of puberty onset.
II	244 patients (174 boys and 70 girls) with DP	Medical history, gonadotropins, testosterone, estradiol, inhibin B, testicular volume, growth velocity, brain MRI	A variety of different diagnoses underlie DP. In boys with DP, prior cryptorchidism, small testicular size, and low inhibin B levels increased the risk for CHH.
III	Growth charts of 36 (27 Finnish and 9 Danish) patients with CHH	Length and weight measurements	Sex steroids appear have an effect on growth during early infancy, since the boys with CHH experienced a reduced growth rate during the first six months of life.
IV	Growth charts of 6 girls with CHH and *FGFR1* mutations	Length and weight measurements	In girls with CHH, no significant length deflection was found during the first six months of life, but the girls showed a decreased height SDS in mid-childhood.
V	30 men with CHH	15D questionnaire	Adult men with CHH experienced an impaired HRQoL, particularly in the dimensions of depression and distress. A history of genital anomalies associated with a reduced HRQoL in the dimension of sexual activity, and the age at diagnosis correlated negatively with the HRQoL.

ISS, idiopathic short stature; Lz, letrozole; Pl, placebo; ELISA, enzyme-linked immunosorbent assay; DP, delayed puberty; CHH; congenital hypogonadotropic hypogonadism; *FGFR1*, fibroblast growth factor receptor 1; SDS, standard deviation score; HRQoL, healt-related quality of life.

DISCUSSION

The exact mechanisms that trigger the onset of puberty are inadequately understood. Similarly, the influence of sex steroids on growth during infancy is unknown. This study addressed these issues by showing for the first time that pubertal activation of the HPG axis is preceded by a decline in serum MKRN3 levels, and that the boys with congenital sex steroid deficiency experience a reduced growth velocity during the first six months of life. Additionally, the reduced growth velocity may serve as a non-reproductive cue to detect boys with CHH. The timely diagnosis of CHH is emphasized by the finding that adult males with CHH experience an impaired HRQoL which associated with the later age of diagnosis. The results also show that a careful review of medical history and physical examination are effective clinical tools in the differential diagnosis of DP, particularly in distinguishing patients with CHH from those with CDGP. The importance of these results is discussed in detail in the subsequent section.

1. Circulating MKRN3 levels in boys before the clinical onset puberty

In boys, circulating MKRN3 levels declined before the onset of puberty, and the decrease was more rapid during prepuberty than after the onset of puberty. Further, the rate of decline in MKRN3 levels associated negatively with the clinical and the biochemical markers of HPG axis activity. Interestingly, after our results were published, a Danish group reported serum MKRN3 levels in healthy peripubertal boys (Busch *et al.* 2016). The study confirmed our findings that in boys MKRN3 levels decline during peripuberty (Busch *et al.* 2016). Conversely, Busch *et al.* reported no correlation between serum MKRN3 levels and the levels of LH, inhibin B or testosterone (Busch *et al.* 2016). This difference may be explained by the fact that circulating MKRN3 exhibited a large inter-individual variation which could have diluted the possible correlations between MKRN3 and biomarkers of puberty (I, (Busch *et al.* 2016)). On the other hand, 16 of our boys with ISS were treated with an AI, which enhances the secretion of gonadotropins and testosterone by removing the negative feedback of estrogen on the HPG axis (**Figure 1A**). This may have resulted in the strengthening of the reciprocal changes between the MKRN3 levels and the biochemical markers of puberty. Altogether, the decline in MKRN3 levels appear to precede the onset of puberty.

The exact mechanisms of MKRN3 in the control of puberty are unclear, albeit it is expressed ubiquitously in human tissue, especially in the testis, (Uhlen *et al.* 2015). *MKRN3* is located at the Prader-Willi and Angelman syndrome critical region in chromosome 15 (Jong *et al.* 1999). MKRN3 is suggested to participate in protein ubiquitination, in which proteins are tagged with an ubiquitin and then send to proteasome and degraded into small peptides (Rock *et al.* 1994). In this process, MKRN3 and other ring zinc-finger proteins tag the target proteins by mediating the transfer on ubiquitin (Schwabe & Klug 1994, Deshaies & Joazeiro 2009). In mice, *Mkrn3* is expressed in the ARC which regulates the activity of GnRH neurons, and the decrease in hypothalamic *Mkrn3* occurs right before the onset of puberty (Abreu *et al.* 2013). In support of this, the zinc-finger protein family appears to control the epigenetic repression of puberty in primates, and the

expression of several zinc-finger protein genes is down-regulated at the time of the HPG reactivation (Lomniczi *et al.* 2015). The levels of MKRN3 in adolescents decline months and yrs before the onset of puberty, which suggests that MKRN3 serves as a transmitter of inhibitory signals to the GnRH neurons (I, (Abreu *et al.* 2013, Hagen *et al.* 2015, Busch *et al.* 2016)). The temporal correlation between the decline in circulating MKRN3, together with the activation of the HPG axis, and the result that MKRN3 levels of boys decline faster before than after the onset of puberty, pinpoints the loosening of the MKRN3 brake to the prepubertal time. The observed inter-individual variation in MKRN3 levels, and the finding that the decline in MKRN3 levels continues with a slower pace throughout the puberty (I, (Busch *et al.* 2016)), suggest the presence of individual MKRN3 threshold for the release of the MKRN3 brake. However, it is noteworthy that MKRN3 is likely not the only regulator of puberty onset, and its functions may be compensated by other factors. Interestingly, circulating MKRN3 levels appear to differ between sexes (Hagen *et al.* 2015, Busch *et al.* 2016), since MKRN3 levels are higher in girls and decline steadier as compared to the biphasic change observed in boys (I, (Hagen *et al.* 2015, Busch *et al.* 2016)). Similarly, CPP is more common in girls than boys ((Abreu *et al.* 2013, Macedo *et al.* 2014), and the boys with CPP and a paternally inherited *MKRN3* mutation experience a smaller decline in the age of puberty onset than the corresponding girls (reviewed in Simon *et al.* 2015). Thus, the role of *MKRN3* in the inhibition of GnRH secretion appear to be more profound in girls.

The absence of evidence describing the relationship between estrogen and MKRN3 is striking. Hagen *et al.* reported longitudinal MKRN3 changes in girls, but they did not find correlation between circulating MKRN3 and estradiol levels (Hagen *et al.* 2015). In contrast, we found that the boys treated with an AI had a slower rate of decline in MKRN3 levels, which suggests that estrogen influences MKRN3 secretion and supports a role of estrogen as a factor that promotes the loosening of the central restrain on the HPG axis (Flor-Cisneros *et al.* 2004, Cui *et al.* 2015). To this end, there appears to be a negative association between a measure of childhood estrogen exposure – bone age – and the age at the onset of puberty (Flor-Cisneros *et al.* 2004). Boys with chronic diseases that have delayed bone maturation, such as malnutrition or GH deficiency, present delayed reactivation of the HPG axis, whereas patients with condition that accelerate bone development, such as CAH, also enter puberty at an earlier age (Flor-Cisneros *et al.* 2004). These findings beg the question if circulating MKRN3 could be used in the differential diagnosis of DP. We found that the boys with late puberty experienced a smaller decline in MKRN3 levels than those with normal timing of puberty, and Hagen *et al.* reported lower levels of MKRN3 in early maturing girls (Hagen *et al.* 2015). However, serum MKRN3 levels in boys are similar to those reported in healthy men and men with CHH (Varimo *et al.* 2016). The effectiveness of serum MKRN3 in detecting disorders of pubertal development should be investigated in a prospective study including adolescents with CDGP, CPP, and CHH.

The studies on circulating MKRN3 levels in humans published so far have shown a large inter-individual variation I, (Hagen *et al.* 2015, Busch *et al.* 2016). The levels of MKRN3 differ between laboratories (Hagen *et al.* 2015, Busch *et al.* 2016, Varimo *et al.* 2016), albeit all the studies in humans have used the same commercial ELISA kit. Consequently, the use of MKRN3 ELISA kit needs to be

validated and replicated in larger cohorts. Additionally, international age- and sex-specific reference standards for circulating MKRN3 are required.

2. The diagnoses and predictors of DP

A wide spectrum of different diagnoses were found to cause DP, of which CDGP was the most common cause in both sexes. The results are largely consistent with the findings of previous studies on DP (Sedlmeyer & Palmert 2002, Reindollar & McDonough 1981, Lawaetz *et al.* 2015, Toublanc, Roger & Chaussain 1991). In our series, however, the frequency of CDGP was higher, whereas the proportions of PHH and FHH were lower than reported in the most comprehensive series of DP published to date (Sedlmeyer & Palmert 2002). The latter difference may be explained by the fact that the study of Sedlmeyer *et al*., as well as most of the previous studies on DP, have been conducted in tertiary centers which may have increased the frequency of rare conditions that underlie DP such as PHH (Sedlmeyer & Palmert 2002, Reindollar & McDonough 1981, Lawaetz *et al.* 2015). This may have resulted in a higher proportion of patients with PHH (Sedlmeyer & Palmert 2002). A recent review, which included a small retrospective evaluation of patients investigated for DP during a two-year period in an academic center, reported a frequency of CDGP (70%) very similar to ours (Abitbol, Zborovski & Palmert 2016). Moreover, the authors of the review speculate that the true number of CDGP patients is probably even higher due to the fact that previous studies have included patients evaluated in a specialized tertiary center (Abitbol, Zborovski & Palmert 2016). Indeed, the high proportion of CDGP in our series might be explained by the patient referral. Our tertiary center outpatient clinic receives direct referrals from general practitioners who evaluate children for pubertal disorders in the Helsinki area school health care. Thus, our series probably resembles the general pediatric population well.

In our series, the proportion of Hyper H was half of the frequency reported before (Sedlmeyer & Palmert 2002). Interestingly, TS was found in 27% of girls with Hyper H, which is a low proportion since TS is reported to affect 1:2500 live births (Stochholm *et al.* 2006), but a frequency that is consistent with the study of Sedlmeyer *et al*. (Sedlmeyer & Palmert 2002). Hence, this suggests that patients with TS are diagnosed earlier than in adolescence with clinical features other than DP, such as reduced growth velocity and short stature. Surprisingly, the proportion of girls with idiopathic ovarian dysfunction was high in our center. The girls tested negative for the Finnish-type FSH receptor gene mutation which causes 29% of cases with primary ovarian failure in Finland (Aittomaki *et al.* 1995, Aittomaki 1994). Since these girls had normal anatomy of the reproductive organs and no ovarian auto-antibodies, this suggests that they may harbor a novel gene defect which results in ovarian dysfunction that should be further studied.

Physicians in school healthcare, pediatricians, and pediatric subspecialists evaluate patients with DP. Performing a wide range of diagnostic tests at the initial evaluation may not always be necessary (Palmert & Dunkel 2012, Abitbol, Zborovski & Palmert 2016). Our results on the outcome predictors of DP emphasize the importance of a careful review of medical history. A history of cryptorchidism was a risk factor for CHH, whereas normally descended testes decreased the risk of permanent hypogonadism. The OR of normally descended testes decreased when it was combined with the knowledge of pubertal timing in

the patient's family. Thus, a boy with normally descended testes who reported DP in a close relative, was likely to have CDGP or FHH as the cause of DP. Further, if these boys do not report any neurological or visual symptoms or findings, the second-line investigations of DP, if needed at all, can be focused on excluding chronic diseases that delay puberty (*i.e.* FHH) and to those fail to show spontaneous pubertal development during follow-up.

Although the annual growth velocity less than 3 cm was associated with the increased frequency of FHH in boys, the further analyses of growth velocity failed to reveal any effective threshold for identifying patients with a pathological underlying cause for DP. However, this may result from the fact that our series included only a few patients with a hypothalamic or pituitary lesion. In girls with DP, the previously reported growth velocity cutoff level (3 cm/yr) performs inadequeately (Palmert & Dunkel 2012). Growth velocity did not prove to be useful in pinpointing the patients who require a brain MRI either. This is important since the patients with lesions in the hypothalamic or pituitary area of CNS show reduced growth velocity long before any neuro-ophthalmic symptoms or signs of delayed puberty are present (Taylor *et al.* 2012). The results of the clinical and the biochemical predictors of DP are summarized in Table 9 (**Table 9**).

Table 9. Clinical and hormonal markers which suggest the diagnostic category of delayed puberty (II).

Predictor	CHH/PHH	CDGP	FHH
History of cryptorchidism	+++		
Heigh velocity < 3 cm/yr			++
Small testicles (< 1.1 mL)	++	+	+
LH < 0.5 IU/L	++	+	+
FSH < 1.2 IU/L	++	+	+
Inhibin B < 60 ng/L	++	+	+
GnRH-induced LH < 4.3 IU/L	++	+	+
TV < 1 mL and inhibin B < 50 ng/L	+++	+	

PHH, permanent hypogonadotropic hypogonadism; CHH, congenital hypogonadotropic hypogonadism; CDGP, constitutional delay of growth and puberty; FHH, functional hypogonadotropic hypogonadism; TV, testicular volume.

The differential diagnosis between CHH and CDGP is extremely challenging, and new evidence-based clinical parameters that could aid in this process are warranted (Harrington & Palmert 2012). Testis size, measured with a ruler, inhibin B and GnRH-induced LH levels emerged as the most effective markers to discriminate between the prepubertal boys with CHH and those with CDGP. After testis size was combined with inhibin B or GnRH-induced LH levels, a slightly improved discriminatory power was reached. Sertoli cell markers, GnRH and hCG stimulation tests, and their combinations have been investigated before in order to find an effective diagnostic parameter that would identify patients with CHH. Unfortunately, none of the tests show a complete accuracy (Harrington & Palmert 2012, Segal *et al.* 2009, Dunkel *et al.* 1985, Coutant *et al.* 2010, Adan *et al.* 2010,

Grinspon *et al.* 2010). We addressed the dilemma from a new viewpoint, and developed a testis size and inhibin B-based clinical prediction tool to estimate the risk of CHH in a prepubertal boy with DP. In the model, subject's inhibin B value is classified in one of the three inhibin B categories obtained from QC measurements instead of using a single inhibin B cutoff level (Coutant *et al.* 2010, Rohayem *et al.* 2015). This is crucial since we know that the performance of inhibin B assay is not optimal at low analyte levels (Kalra *et al.* 2010). The prediction model may serve as an additional support for the clinician pondering between CHH and CDGP.

3. The influence of sex steroid deficiency on growth during minipuberty, childhood and adolescence

We depicted the growth charts of patients with CHH from birth to adult height. During minipuberty, the boys with CHH experienced a transient deflection in the length SDS, whereas a similar decrease was not evident in girls. The findings suggest that the minipubertal length deflection is a new non-reproductive feature of CHH, and that androgens modulate growth during the first six months of life. Importantly, we found that the decline in length SDS was more profound in boys with signs of a severe GnRH deficiency (*i.e.* a history of cryptorchidism or micropenis). In line with our finding, minipubertal testosterone levels in healthy boys correlate positively with penile growth and length velocity (Boas *et al.* 2006, Kiviranta *et al.* 2016). Boys have a more rapid growth velocity than girls during the first six months of life (Kiviranta *et al.* 2016), which may be explained by a sex-difference in hormonal milieu. During minipuberty, testosterone levels rise rapidly in boys, whereas in girls estradiol levels increase, but fluctuate and decline more slowly (reviewed in Kuiri-Hanninen, Sankilampi & Dunkel 2014). Thus, it would be tempting to postulate that, in boys, the growth during early infancy is more sensitive to the influence of sex-steroids. Nevertheless, the importance of nutrition in growth during early infancy cannot be excluded. During the first year of life, liver IGF-1 secretion is nutrition-dependent and carbohydrate-containing diet associates positively with growth rate (Socha *et al.* 2011, Chellakooty *et al.* 2006). The information on the nutrition during minipuberty was not available in our patients due to the retrospective setting. The study included the growth measurements of only six girls with CHH, thus, some changes in growth may have escaped detection. However, the findings in healthy boys treated with testosterone for micropenis, strongly support the role of androgens in the modulation of growth during early infancy (Landier, Chaussain & Job 1984, Guthrie, Smith & Graham 1973).

Both the boys and the girls with CHH experienced a relatively normal childhood growth, which is consistent with the sparse growth data of CHH patients published before (Van Dop *et al.* 1987, Raboch & Reisenauer 1976, Dickerman, Cohen & Laron 1992, Kaushanski & Laron 1979). The decreased growth rate observed in patients with CHH at the age of 4 to 7 yrs supports the view that estrogen regulates bone and linear growth already during prepuberty (Cutler 1997, Janner, Fluck & Mullis 2012). In both sexes, the height SDS reached its nadir right before the induction of puberty.

The initiation of sex steroid treatment recovered the linear growth, since the adult heights were similar to MPHs. In contrast, previous studies have reported

that patients with CHH usually exceed their MPHs and adult heights of the general population due to the delayed fusion of the epiphyseal plates (Raboch & Reisenauer 1976, Dickerman, Cohen & Laron 1992, Uriarte *et al.* 1992). This difference may be explained by the findings that the average age of puberty induction was lower in our study than reported before (Raboch & Reisenauer 1976, Dickerman, Cohen & Laron 1992, Uriarte *et al.* 1992), and the secular trend in the age of puberty induction in Finland had declined from the 1970s onwards. Since the cohort extended as far as the 1970s, there were patients who received very high doses of testosterone to induce pubertal maturation at the expense of losing some of their growth potential. Indeed, we found that the cumulative dosage of testosterone during the first treatment year correlated negatively with the adult height. Altogether, these results underline the importance of timely diagnosis and treatment of CHH.

The influence of different genotypes to linear growth remain speculative. We found no association between molecular genetic diagnosis and growth during infancy, childhood or adult height. The patients with CHH and a verified gene mutation experienced weight gain during infancy, childhood, and adolescence, which was similar to those without gene mutations. In fact, the only difference found was that the boys with *ANOS1* were shorter than those with *FGFR1* mutations at birth. The further investigation of this genotype-growth correlation is hindered by the absence of *anos1* mouse model. However, since an excess androgen milieu during fetal life results in a higher birth length (Balsamo *et al.* 2006) and *ANOS1* usually relates to a more severe gonadotropin and sex steroid deficiency (Costa-Barbosa *et al.* 2013), it could be speculated that the lower birth length in patients with *ANOS1* stems from the altered intrauterine sex steroid milieu.

4. Health-related quality of life in males with CHH

Congenital sex steroid deficiency associated with an impaired HRQoL in adulthood. Specifically, the males with CHH reported lower HRQoL scores than the age- and sex-matched sample of general population, and the dimensions mostly affected were depression and distress. This is in agreement with the two previous studies on HRQoL in patients with CHH (Aydogan *et al.* 2012, Shiraishi, Oka & Matsuyama 2014), and it supports the view that congenital sex steroid deficiency causes long-term consequences on general well-being in adulthood. Remarkably, the HRQoL dimensions affected in our cohort were similar to those reported in the study by Aydogan *et al.* (Aydogan *et al.* 2012). In the previous studies, hormone replacement therapy improved significantly several domains of HRQoL, especially the dimensions related to psychological well-being (Aydogan *et al.* 2012, Shiraishi, Oka & Matsuyama 2014). This supports a causative relationship between long-term testosterone deficiency and impaired HRQoL. In contrast, we found no association between the adequate testosterone replacement therapy during adulthood and HRQoL scores. The results may be explained by the cross-sectional design of our study, or by the fact that the primary effects of delayed physical changes of puberty occur already during adolescence and early adulthood, before the onset of testosterone therapy, and paves the way for the long-lasting psychosocial consequences. In support of this, we found a negative correlation between the age at diagnosis and the HRQoL scores, and that CHH men with a history genital anomalies (micropenis and/or cryptorchidism)

have experienced a lower HRQoL in the dimension of sexual activity. A recent survey, which included males with CHH, identified negative psychological effects attributed to late diagnosis of CHH (Dwyer *et al.* 2014). In particular, the patients expressed anxiety and depression, body image concerns, low self-esteem, and a sensation of being left-behind as their peers developed into adult bodies (Dwyer *et al.* 2014). Although the data are limited, these findings support the concept that the timely diagnosis of males with CHH is vital for the prevention of long-term psychological consequences.

5. Future perspectives

The mechanisms that trigger the onset of puberty remain elusive. The discovery of *MKRN3* as one of the gatekeepers of pubertal onset, has focused the research on finding novel genes that contribute to the timing of puberty. Similarly, the exact role of extrogen in this process is unknown. At the same time, clinicians who evaluate adolescents with DP face not only the problem of identifying patients with pathological underlying cause, but also selecting right patients for the second-line investigations. Thus, the need for new evidence-based clinical tools to aid in this process is evident.

Late puberty can cause significant psychosocial burden to adolescents, especially to boys, and even limit their social integration with peers (Gross & Duke 1980). Currently, the boys with CDGP have two options for the management of late puberty in Finland: watchful waiting and expediting pubertal changes with a low-dose intramuscular testosterone. The dose and the treatment period of low-dose testosterone vary (Ambler 2009). Moreover, intra-muscular testosterone appears to suppress rather than activate the HPG axis, which questions its role as a standard of care for CDGP (Palmert & Dunkel 2012, Ambler 2009). Interestingly, the studies in boys with ISS have shown that AIs stimulate endogenous gonadotropin and testosterone production, and augment the progression of puberty (Dunkel & Wickman 2001, Hero, Norjavaara & Dunkel 2005). In an attempt to compare AIs to the current standard of care in the DP, this needs to be evaluated in a randomized-controlled setting, such as the one currently ongoing in five Finnish hospitals.

ACKNOWLEDGEMENTS

This doctoral thesis was carried out between 2013 and 2016 at the Children's Hospital, the Helsinki University hospital, and the University of Helsinki. I would like to express my gratitude to Docent Jari Petäjä (director of department of pediatrics), Professor Markku Heikinheimo (Head of Children's Hospital and Institute of Clinical Medicine), Professor Mikael Knip (Head of Institute of Clinical Medicine), and Docent Jussi Merenmies (Head of the Pediatric Graduate School) for providing excellent research facilities.

This study was financially supported by the Finnish Medical Foundation, Foundation for Pediatric Research, and University of Helsinki. I warmly thank all the participants who took part in the studies and therefore made this thesis possible.

I am very grateful to my two outstanding supervisors; Professor Taneli Raivio and MD, PhD Matti Hero. Thank you for offering the opportunity to work with you. Your support, and the fact that you always had time to talk over matters occypying my mind, have been reassuring. I feel that I have learned very much from both of you during this project. You have planted a dawning interest of clinical research in me, which will not vanish with the completion of this thesis. Taneli, I admire your relentless enthusiasm and expertise which covers both the clinical practice and research. Your endurance in finalizing a manuscript is inspiring. Thank you for your friendship and for encouraging me to excel myself many times during this project. Matti, I am very grateful for your friendship and the opportunity of learning from you. Our conversations on many research topics, such as the basis of human growth, have been an eye-opening experience for me. Your dedication in pediatric research and clinical work are admirable.

In addition to my supervisors, I would like to present my gratitude to Docent Päivi Miettinen who has had a significant contribution to this project. Thank you for our guidance and reasoning during this project.

I also wish to thank the members of my thesis committee. Professor Juha Tapanainen, thank you for your support and also being the official reviewer of this thesis. Docent Krista Rantakari thank you for the constructive conversations and guidance during this project. Similarly, I would like express my gratitude to Docent Marja Ojaniemi for closely reviewing this study and for providing valuable and constructive critic on it.

During the last four years I have struggled to balance my time between research work and clinical practice. Thus, I would like to express my gratitude to my mentors in clinical work as well. The first person to introduce me to the interesting world of pediatrics was my uncle Pertti. Thank you for inspiring me to begin this journey in Kajaani. Also, I would like to thank the chief of staff Arja Tomminen at Kanta-Häme central hospital for supporting and guiding me during the time I worked there. Arja, I am especially grateful for the encouragement to start research work alongside with the clinical practice. In addition, I want to thank Mikko Huhtanen for the guidance and bedside teaching. I owe special gratitude to Anssi Luoma for the relentless mentoring at the beginning of my pediatric career. Your exceptional humor and wit spiced up even the most boring days! Not to forget the fact that you always thought what was the best for the sick children.

I would like to thank my fellow researchers Jenni, Juuso, Johanna S, Heta, Laura, Ella, Kirsi, and Johanna K for sharing the pleasant and not so pleasant moments of research work. Also, I would like to thank Annika for the linguistic guidance in this project.

Being a coordinating investigator in a clinical drug trial has taken a significant amount of my resources alongside the thesis. This required me to work closely with the staff of the day hospital and the pediatric endocrine outpatient clinic at the Children's Hospital. Thus, I would like to show my gratitude to the outstanding staff of these clinics.

Outside work, I would like to thank my lifelong friends Ilkka and Marko for being there for me starting from the comprehensive school. I owe gratitude to my dear friends Ruut and Leevi, Lydia and Matti, and Minna and Matti for your friendship and constructive conversations.

This project would not have been completed without the support of my family. I would like to express my sincere gratitude to my dear brother Vesa, and sister-in-law Inari. Thank you for your support and encouragement. Also, I would like to thank my brother-in-law Aki for your friendship. Your endurance and happiness has been an inspiration to me. Thank you for the numerous relaxing fishing trips which have been enjoyable and a great counterbalance to this project.

My parents-in-law Riitta and Tapio are to be thanked for their endless support. You have been a tremendous help. Without you this project would have never been completed. I am privileged to have two sets of parents.

I would like to show my deepest gratitude to my parents Kaija and Matti. Thank you for everything! Without your unconditional support and encouragement I would have never begun this project, not to mention the career of a physician.

Finally, I would like to thank the two of my most precious things in this world; my son Joonatan and my wife Eveliina. Joonatan, I have enjoyed watching you grow, play, learn, and observe the surroundings. Your limitless imagination and enthusiasm for new things is inspiring. Eveliina, thank you for letting me share this life with you. You have always been there for me and believed in me. I love you!

Tero Varimo
Helsinki, November 2016

REFERENCES

Abbassi V. Growth and normal puberty. *Pediatrics* 1998;102:507-511.

Abitbol L, Zborovski S, Palmert MR. Evaluation of delayed puberty: what diagnostic tests should be performed in the seemingly otherwise well adolescent? *Arch Dis Child* 2016;101:767-771.

Abreu AP, Dauber A, Macedo DB, Noel SD, Brito VN, Gill JC, Cukier P, Thompson IR, Navarro VM, Gagliardi PC, Rodrigues T, Kochi C, Longui CA, Beckers D, de Zegher F, Montenegro LR, Mendonca BB, Carroll RS, Hirschhorn JN, Latronico AC, Kaiser UB. Central precocious puberty caused by mutations in the imprinted gene MKRN3. *N Engl J Med* 2013;368:2467-2475.

Abreu AP, Macedo DB, Brito VN, Kaiser UB, Latronico AC. A new pathway in the control of the initiation of puberty: the MKRN3 gene. *J Mol Endocrinol* 2015;54:R131-9.

Adan L, Lechevalier P, Couto-Silva AC, Boissan M, Trivin C, Brailly-Tabard S, Brauner R. Plasma inhibin B and antimullerian hormone concentrations in boys: discriminating between congenital hypogonadotropic hypogonadism and constitutional pubertal delay. *Med Sci Monit* 2010;16:CR511-7.

Ahlborg HG, Johnell O, Turner CH, Rannevik G, Karlsson MK. Bone loss and bone size after menopause. *N Engl J Med* 2003;349:327-334.

Aittomaki K. The genetics of XX gonadal dysgenesis. *Am J Hum Genet* 1994;54:844-851.

Aittomaki K, Lucena JL, Pakarinen P, Sistonen P, Tapanainen J, Gromoll J, Kaskikari R, Sankila EM, Lehvaslaiho H, Engel AR, Nieschlag E, Huhtaniemi I, de la Chapelle A. Mutation in the follicle-stimulating hormone receptor gene causes hereditary hypergonadotropic ovarian failure. *Cell* 1995;82:959-968.

Aksglaede L, Sorensen K, Boas M, Mouritsen A, Hagen CP, Jensen RB, Petersen JH, Linneberg A, Andersson AM, Main KM, Skakkebaek NE, Juul A. Changes in anti-Mullerian hormone (AMH) throughout the life span: a population-based study of 1027 healthy males from birth (cord blood) to the age of 69 years. *J Clin Endocrinol Metab* 2010;95:5357-5364.

Aksglaede L, Sorensen K, Petersen JH, Skakkebaek NE, Juul A. Recent decline in age at breast development: the Copenhagen Puberty Study. *Pediatrics* 2009;123:e932-9.

Alanne S, Roine RP, Rasanen P, Vainiola T, Sintonen H. Estimating the minimum important change in the 15D scores. *Qual Life Res* 2015;24:599-606.

Albertsson-Wikland K, Rosberg S, Lannering B, Dunkel L, Selstam G, Norjavaara E. Twenty-four-hour profiles of luteinizing hormone, follicle-stimulating hormone, testosterone, and estradiol levels: a semilongitudinal study throughout puberty in healthy boys. *J Clin Endocrinol Metab* 1997;82:541-549.

Albin AK, Niklasson A, Westgren U, Norjavaara E. Estradiol and pubertal growth in girls. *Horm Res Paediatr* 2012;78:218-225.

Ambler GR. Androgen therapy for delayed male puberty. *Curr Opin Endocrinol Diabetes Obes* 2009;16:232-239.

Andersson AM, Juul A, Petersen JH, Muller J, Groome NP, Skakkebaek NE. Serum inhibin B in healthy pubertal and adolescent boys: relation to age, stage of puberty, and follicle-stimulating hormone, luteinizing hormone, testosterone, and estradiol levels. *J Clin Endocrinol Metab* 1997;82:3976-3981.

Andersson AM, Petersen JH, Jorgensen N, Jensen TK, Skakkebaek NE. Serum inhibin B and follicle-stimulating hormone levels as tools in the evaluation of infertile men: significance of adequate reference values from proven fertile men. *J Clin Endocrinol Metab* 2004;89:2873-2879.

Andersson AM, Toppari J, Haavisto AM, Petersen JH, Simell T, Simell O, Skakkebaek NE. Longitudinal reproductive hormone profiles in infants: peak of inhibin B levels in infant boys exceeds levels in adult men. *J Clin Endocrinol Metab* 1998;83:675-681.

Ankarberg-Lindgren C, Kristrom B, Norjavaara E. Physiological estrogen replacement therapy for puberty induction in girls: a clinical observational study. *Horm Res Paediatr* 2014;81:239-244.

Ankarberg-Lindgren C, Norjavaara E. Changes of diurnal rhythm and levels of total and free testosterone secretion from pre to late puberty in boys: testis size of 3 ml is a transition stage to puberty. *Eur J Endocrinol* 2004;151:747-757.

Apter D, Butzow TL, Laughlin GA, Yen SS. Gonadotropin-releasing hormone pulse generator activity during pubertal transition in girls: pulsatile and diurnal patterns of circulating gonadotropins. *J Clin Endocrinol Metab* 1993;76:940-949.

Aydogan U, Aydogdu A, Akbulut H, Sonmez A, Yuksel S, Basaran Y, Uzun O, Bolu E, Saglam K. Increased frequency of anxiety, depression, quality of life and sexual life in young hypogonadotropic hypogonadal males and impacts of testosterone replacement therapy on these conditions. *Endocr J* 2012;59:1099-1105.

Bailleul-Forestier I, Gros C, Zenaty D, Bennaceur S, Leger J, de Roux N. Dental agenesis in Kallmann syndrome individuals with FGFR1 mutations. *Int J Paediatr Dent* 2010;20:305-312.

Balsamo A, Wasniewska M, Di Pasquale G, Salzano G, Baronio F, Bombaci S, De Luca F. Birth length and weight in congenital adrenal hyperplasia according to the different phenotypes. *Eur J Pediatr* 2006;165:380-383.

Baumann G. Growth hormone binding protein 2001. *J Pediatr Endocrinol Metab* 2001;14:355-375.

Bayley N, Pinneau SR. Tables for predicting adult height from skeletal age: revised for use with the Greulich-Pyle hand standards. *J Pediatr* 1952;40:423-441.

Behre HM, Kliesch S, Leifke E, Link TM, Nieschlag E. Long-term effect of testosterone therapy on bone mineral density in hypogonadal men. *J Clin Endocrinol Metab* 1997;82:2386-2390.

Behre HM, Nashan D, Nieschlag E. Objective measurement of testicular volume by ultrasonography: evaluation of the technique and comparison with orchidometer estimates. *Int J Androl* 1989;12:395-403.

Bennett GW, Edwardson JA, Holland D, Jeffcoate SL, White N. Release of immunoreactive luteinising hormone-releasing hormone and thyrotrophin-releasing hormone from hypothalamus. *Nature* 1975;257:323-325.

Bergada I, Bergada C. Long term treatment with low dose testosterone in constitutional delay of growth and puberty: effect on bone age maturation and pubertal progression. *J Pediatr Endocrinol Metab* 1995;8:117-122.

Bergada I, Milani C, Bedecarras P, Andreone L, Ropelato MG, Gottlieb S, Bergada C, Campo S, Rey RA. Time course of the serum gonadotropin surge, inhibins, and anti-Mullerian hormone in normal newborn males during the first month of life. *J Clin Endocrinol Metab* 2006;91:4092-4098.

Bertelloni S, Mul D. Treatment of central precocious puberty by GnRH analogs: long-term outcome in men. *Asian J Androl* 2008;10:525-534.

Bianco SD, Kaiser UB. The genetic and molecular basis of idiopathic hypogonadotropic hypogonadism. *Nat Rev Endocrinol* 2009;5:569-576.

Binder G, Schweizer R, Blumenstock G, Braun R. Inhibin B plus LH vs GnRH agonist test for distinguishing constitutional delay of growth and puberty from isolated hypogonadotropic hypogonadism in boys. *Clin Endocrinol (Oxf)* 2015;82:100-105.

Biro FM, Greenspan LC, Galvez MP, Pinney SM, Teitelbaum S, Windham GC, Deardorff J, Herrick RL, Succop PA, Hiatt RA, Kushi LH, Wolff MS. Onset of breast development in a longitudinal cohort. *Pediatrics* 2013;132:1019-1027.

Blake CA, Sridaran R, Elias KA, Ashiru OA, Rush ME. Plasma LH patterns after LHRH infusion in long-term, unanesthetized ovariectomized rats. Evidence for neural control of the pulsatile LH phenomenon. *Neuroendocrinology* 1980;30:45-51.

Bless E, Raitcheva D, Henion TR, Tobet S, Schwarting GA. Lactosamine modulates the rate of migration of GnRH neurons during mouse development. *Eur J Neurosci* 2006;24:654-660.

Boas M, Boisen KA, Virtanen HE, Kaleva M, Suomi AM, Schmidt IM, Damgaard IN, Kai CM, Chellakooty M, Skakkebaek NE, Toppari J, Main KM. Postnatal penile length and growth rate correlate to serum testosterone levels: a longitudinal study of 1962 normal boys. *Eur J Endocrinol* 2006;154:125-129.

Boehm U, Bouloux PM, Dattani MT, de Roux N, Dode C, Dunkel L, Dwyer AA, Giacobini P, Hardelin JP, Juul A, Maghnie M, Pitteloud N, Prevot V, Raivio T, Tena-Sempere M, Quinton R, Young J. Expert consensus document: European Consensus Statement on congenital hypogonadotropic hypogonadism--pathogenesis, diagnosis and treatment. *Nat Rev Endocrinol* 2015;11:547-564.

Boepple PA, Hayes FJ, Dwyer AA, Raivio T, Lee H, Crowley WF,Jr, Pitteloud N. Relative roles of inhibin B and sex steroids in the negative feedback regulation of follicle-stimulating hormone in men across the full spectrum of seminiferous epithelium function. *J Clin Endocrinol Metab* 2008;93:1809-1814.

Bolton NJ, Tapanainen J, Koivisto M, Vihko R. Circulating sex hormone-binding globulin and testosterone in newborns and infants. *Clin Endocrinol (Oxf)* 1989;31:201-207.

Bonjour JP, Chevalley T. Pubertal timing, bone acquisition, and risk of fracture throughout life. *Endocr Rev* 2014;35:820-847.

Bouligand J, Ghervan C, Tello JA, Brailly-Tabard S, Salenave S, Chanson P, Lombes M, Millar RP, Guiochon-Mantel A, Young J. Isolated familial hypogonadotropic hypogonadism and a GNRH1 mutation. *N Engl J Med* 2009;360:2742-2748.

Bouvattier C, Maione L, Bouligand J, Dode C, Guiochon-Mantel A, Young J. Neonatal gonadotropin therapy in male congenital hypogonadotropic hypogonadism. *Nat Rev Endocrinol* 2011;8:172-182.

Burgus R, Butcher M, Ling N, Monahan M, Rivier J, Fellows R, Amoss M, Blackwell R, Vale W, Guillemin R. Molecular structure of the hypothalamic factor (LRF) of ovine origin monitoring the secretion of pituitary gonadotropic hormone of luteinization (LH). *C R Acad Sci Hebd Seances Acad Sci D* 1971;273:1611-1613.

Busch AS, Hagen CP, Almstrup K, Juul A. Circulating MKRN3 Levels Decline During Puberty in Healthy Boys. *J Clin Endocrinol Metab* 2016;101:2588-2593.

Canale D, Vignali E, Golia F, Martino E, Pinchera A, Marcocci C. Effects of hormonal replacement treatment on bone mineral density and metabolism in hypogonadal patients. *Mol Cell Endocrinol* 2000;161:47-51.

Carani C, Qin K, Simoni M, Faustini-Fustini M, Serpente S, Boyd J, Korach KS, Simpson ER. Effect of testosterone and estradiol in a man with aromatase deficiency. *N Engl J Med* 1997;337:91-95.

Carel JC, Leger J. Clinical practice. Precocious puberty. *N Engl J Med* 2008;358:2366-2377.

Casoni F, Hutchins BI, Donohue D, Fornaro M, Condie BG, Wray S. SDF and GABA interact to regulate axophilic migration of GnRH neurons. *J Cell Sci* 2012;125:5015-5025.

Chan YM, Lippincott MF, Butler JP, Sidhoum VF, Li CX, Plummer L, Seminara SB. Exogenous kisspeptin administration as a probe of GnRH neuronal function in patients with idiopathic hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 2014;99:E2762-71.

Chellakooty M, Juul A, Boisen KA, Damgaard IN, Kai CM, Schmidt IM, Petersen JH, Skakkebaek NE, Main KM. A prospective study of serum insulin-like growth factor I (IGF-I) and IGF-binding protein-3 in 942 healthy infants: associations with birth weight, gender, growth velocity, and breastfeeding. *J Clin Endocrinol Metab* 2006;91:820-826.

Chemes HE, Rey RA, Nistal M, Regadera J, Musse M, Gonzalez-Peramato P, Serrano A. Physiological androgen insensitivity of the fetal, neonatal, and early infantile testis is explained by the ontogeny of the androgen receptor expression in Sertoli cells. *J Clin Endocrinol Metab* 2008;93:4408-4412.

Chung WC, Tsai PS. Role of fibroblast growth factor signaling in gonadotropin-releasing hormone neuronal system development. *Front Horm Res* 2010;39:37-50.

Clarkson J, Han SK, Liu X, Lee K, Herbison AE. Neurobiological mechanisms underlying kisspeptin activation of gonadotropin-releasing hormone (GnRH) neurons at puberty. *Mol Cell Endocrinol* 2010;324:45-50.

Clayton PE, Shalet SM, Price DA, Addison GM. Growth and growth hormone responses to oxandrolone in boys with constitutional delay of growth and puberty (CDGP). *Clin Endocrinol (Oxf)* 1988;29:123-130.

Clements JA, Reyes FI, Winter JS, Faiman C. Studies on human sexual development. III. Fetal pituitary and serum, and amniotic fluid concentrations of LH, CG, and FSH. *J Clin Endocrinol Metab* 1976;42:9-19.

Codesal J, Regadera J, Nistal M, Regadera-Sejas J, Paniagua R. Involution of human fetal Leydig cells. An immunohistochemical, ultrastructural and quantitative study. *J Anat* 1990;172:103-114.

Cole TJ. Secular trends in growth. *Proc Nutr Soc* 2000;59:317-324.

Corbier P, Dehennin L, Castanier M, Mebazaa A, Edwards DA, Roffi J. Sex differences in serum luteinizing hormone and testosterone in the human neonate during the first few hours after birth. *J Clin Endocrinol Metab* 1990;71:1344-1348.

Cortes D, Muller J, Skakkebaek NE. Proliferation of Sertoli cells during development of the human testis assessed by stereological methods. *Int J Androl* 1987;10:589-596.

Costa-Barbosa FA, Balasubramanian R, Keefe KW, Shaw ND, Al-Tassan N, Plummer L, Dwyer AA, Buck CL, Choi JH, Seminara SB, Quinton R, Monies D, Meyer B, Hall JE, Pitteloud N, Crowley WF,Jr.

Prioritizing genetic testing in patients with Kallmann syndrome using clinical phenotypes. *J Clin Endocrinol Metab* 2013;98:E943-53.

Coutant R, Biette-Demeneix E, Bouvattier C, Bouhours-Nouet N, Gatelais F, Dufresne S, Rouleau S, Lahlou N. Baseline inhibin B and anti-Mullerian hormone measurements for diagnosis of hypogonadotropic hypogonadism (HH) in boys with delayed puberty. *J Clin Endocrinol Metab* 2010;95:5225-5232.

Crowley WF,Jr. Commentary: the year in endocrine genetics for basic scientists. *Mol Endocrinol* 2011;25:1989-2002.

Cui P, Yang C, Zhang K, Gao X, Luo L, Tian Y, Song M, Liu Y, Zhang Y, Li Y, Zhang X, Su S, Fang F, Ding J. Effect of estrogen on the expression of GnRH and kisspeptin in the hypothalamus of rats during puberty. *Theriogenology* 2015;84:1556-1564.

Cutler GB,Jr. The role of estrogen in bone growth and maturation during childhood and adolescence. *J Steroid Biochem Mol Biol* 1997;61:141-144.

de Kretser DM, Meinhardt A, Meehan T, Phillips DJ, O'Bryan MK, Loveland KA. The roles of inhibin and related peptides in gonadal function. *Mol Cell Endocrinol* 2000;161:43-46.

de Roux N, Genin E, Carel JC, Matsuda F, Chaussain JL, Milgrom E. Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. *Proc Natl Acad Sci U S A* 2003;100:10972-10976.

de Roux N, Young J, Misrahi M, Genet R, Chanson P, Schaison G, Milgrom E. A family with hypogonadotropic hypogonadism and mutations in the gonadotropin-releasing hormone receptor. *N Engl J Med* 1997;337:1597-1602.

Debieve F, Beerlandt S, Hubinont C, Thomas K. Gonadotropins, prolactin, inhibin A, inhibin B, and activin A in human fetal serum from midpregnancy and term pregnancy. *J Clin Endocrinol Metab* 2000;85:270-274.

Decourt C, Robert V, Anger K, Galibert M, Madinier JB, Liu X, Dardente H, Lomet D, Delmas AF, Caraty A, Herbison AE, Anderson GM, Aucagne V, Beltramo M. A synthetic kisspeptin analog that triggers ovulation and advances puberty. *Sci Rep* 2016;6:26908.

Della Valle E, Vezzani S, Rochira V, Granata AR, Madeo B, Genovese E, Pignatti E, Marino M, Carani C, Simoni M. Prevalence of olfactory and other developmental anomalies in patients with central hypogonadotropic hypogonadism. *Front Endocrinol (Lausanne)* 2013;4:70.

Denzer C, Weibel A, Muche R, Karges B, Sorgo W, Wabitsch M. Pubertal development in obese children and adolescents. *Int J Obes (Lond)* 2007;31:1509-1519.

Deshaies RJ, Joazeiro CA. RING domain E3 ubiquitin ligases. *Annu Rev Biochem* 2009;78:399-434.

Diamond DA, Paltiel HJ, DiCanzio J, Zurakowski D, Bauer SB, Atala A, Ephraim PL, Grant R, Retik AB. Comparative assessment of pediatric testicular volume: orchidometer versus ultrasound. *J Urol* 2000;164:1111-1114.

Dickerman Z, Cohen A, Laron Z. Growth in patients with isolated gonadotrophin deficiency. *Arch Dis Child* 1992;67:513-516.

Dickerman Z, Prager-Lewin R, Laron Z. The plasma FSH and LH response to synthetic LH-RH in normal pre-pubertal and early pubertal girls--a re-evaluation. *Acta Endocrinol (Copenh)* 1979;91:14-18.

Dode C, Levilliers J, Dupont JM, De Paepe A, Le Du N, Soussi-Yanicostas N, Coimbra RS, Delmaghani S, Compain-Nouaille S, Baverel F, Pecheux C, Le Tessier D, Cruaud C, Delpech M, Speleman F, Vermeulen S, Amalfitano A, Bachelot Y, Bouchard P, Cabrol S, Carel JC, Delemarre-van de Waal H, Goulet-Salmon B, Kottler ML, Richard O, Sanchez-Franco F, Saura R, Young J, Petit C, Hardelin JP. Loss-of-function mutations in FGFR1 cause autosomal dominant Kallmann syndrome. *Nat Genet* 2003;33:463-465.

Dode C, Teixeira L, Levilliers J, Fouveaut C, Bouchard P, Kottler ML, Lespinasse J, Lienhardt-Roussie A, Mathieu M, Moerman A, Morgan G, Murat A, Toublanc JE, Wolczynski S, Delpech M, Petit C, Young J, Hardelin JP. Kallmann syndrome: mutations in the genes encoding prokineticin-2 and prokineticin receptor-2. *PLoS Genet* 2006;2:e175.

Doneray H, Orbak Z. Association between anthropometric hormonal measurements and bone mineral density in puberty and constitutional delay of growth and puberty. *West Indian Med J* 2010;59:125-130.

Dorling AA, Todman MG, Korach KS, Herbison AE. Critical role for estrogen receptor alpha in negative feedback regulation of gonadotropin-releasing hormone mRNA expression in the female mouse. *Neuroendocrinology* 2003;78:204-209.

Dubois SL, Acosta-Martinez M, DeJoseph MR, Wolfe A, Radovick S, Boehm U, Urban JH, Levine JE. Positive, but not negative feedback actions of estradiol in adult female mice require estrogen receptor alpha in kisspeptin neurons. *Endocrinology* 2015;156:1111-1120.

Dubois SL, Wolfe A, Radovick S, Boehm U, Levine JE. Estradiol Restrains Prepubertal Gonadotropin Secretion in Female Mice via Activation of ERalpha in Kisspeptin Neurons. *Endocrinology* 2016;157:1546-1554.

Dunkel L, Perheentupa J, Virtanen M, Maenpaa J. Gonadotropin-releasing hormone test and human chorionic gonadotropin test in the diagnosis of gonadotropin deficiency in prepubertal boys. *J Pediatr* 1985;107:388-392.

Dunkel L, Quinton R. Transition in endocrinology: induction of puberty. *Eur J Endocrinol* 2014;170:R229-39.

Dunkel L, Wickman S. Treatment of delayed male puberty: efficacy of aromatase inhibition. *J Pediatr Endocrinol Metab* 2001;14 Suppl 6:1541-1546.

Dwyer AA, Jayasena CN, Quinton R. Congenital hypogonadotropic hypogonadism: implications of absent mini-puberty. *Minerva Endocrinol* 2016;41:188-195.

Dwyer AA, Phan-Hug F, Hauschild M, Elowe-Gruau E, Pitteloud N. TRANSITION IN ENDOCRINOLOGY: Hypogonadism in adolescence. *Eur J Endocrinol* 2015;173:R15-24.

Dwyer AA, Quinton R, Morin D, Pitteloud N. Identifying the unmet health needs of patients with congenital hypogonadotropic hypogonadism using a web-based needs assessment: implications for online interventions and peer-to-peer support. *Orphanet J Rare Dis* 2014;9:83-1172-9-83.

Elks CE, Perry JR, Sulem P, Chasman DI, Franceschini N, He C, Lunetta KL, Visser JA, Byrne EM, Cousminer DL, Gudbjartsson DF, Esko T, Feenstra B, Hottenga JJ, Koller DL, Kutalik Z, Lin P, Mangino M, Marongiu M, McArdle PF, Smith AV, Stolk L, van Wingerden SH, Zhao JH, Albrecht E, Corre T, Ingelsson E, Hayward C, Magnusson PK, Smith EN, Ulivi S, Warrington NM, Zgaga L, Alavere H, Amin N, Aspelund T, Bandinelli S, Barroso I, Berenson GS, Bergmann S, Blackburn H, Boerwinkle E, Buring JE, Busonero F, Campbell H, Chanock SJ, Chen W, Cornelis MC, Couper D, Coviello AD, d'Adamo P, de Faire U, de Geus EJ, Deloukas P, Doring A, Smith GD, Easton DF, Eiriksdottir G, Emilsson V, Eriksson J, Ferrucci L, Folsom AR, Foroud T, Garcia M, Gasparini P, Geller F, Gieger C, GIANT Consortium, Gudnason V, Hall P, Hankinson SE, Ferreli L, Heath AC, Hernandez DG, Hofman A, Hu FB, Illig T, Jarvelin MR, Johnson AD, and collaborators. Thirty new loci for age at menarche identified by a meta-analysis of genome-wide association studies. *Nat Genet* 2010;42:1077-1085.

Ersoy B, Balkan C, Gunay T, Egemen A. The factors affecting the relation between the menarcheal age of mother and daughter. *Child Care Health Dev* 2005;31:303-308.

Euling SY, Herman-Giddens ME, Lee PA, Selevan SG, Juul A, Sorensen TI, Dunkel L, Himes JH, Teilmann G, Swan SH. Examination of US puberty-timing data from 1940 to 1994 for secular trends: panel findings. *Pediatrics* 2008;121 Suppl 3:S172-91.

Finkelstein JS, Klibanski A, Neer RM, Doppelt SH, Rosenthal DI, Segre GV, Crowley WF,Jr. Increases in bone density during treatment of men with idiopathic hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 1989;69:776-783.

Finkelstein JS, O'Dea LS, Whitcomb RW, Crowley WF,Jr. Sex steroid control of gonadotropin secretion in the human male. II. Effects of estradiol administration in normal and gonadotropin-releasing hormone-deficient men. *J Clin Endocrinol Metab* 1991;73:621-628.

Fintini D, Brufani C, Grossi A, Ubertini G, Fiori R, Pecorelli L, Calzolari A, Cappa M. Gender differences in bone mineral density in obese children during pubertal development. *J Endocrinol Invest* 2011;34:e86-91.

Flor-Cisneros A, Leschek EW, Merke DP, Barnes KM, Coco M, Cutler GB,Jr, Baron J. In boys with abnormal developmental tempo, maturation of the skeleton and the hypothalamic-pituitary-gonadal axis remains synchronous. *J Clin Endocrinol Metab* 2004;89:236-241.

Forest MG, Sizonenko PC, Cathiard AM, Bertrand J. Hypophyso-gonadal function in humans during the first year of life. 1. Evidence for testicular activity in early infancy. *J Clin Invest* 1974;53:819-828.

Forni PE, Taylor-Burds C, Melvin VS, Williams T, Wray S. Neural crest and ectodermal cells intermix in the nasal placode to give rise to GnRH-1 neurons, sensory neurons, and olfactory ensheathing cells. *J Neurosci* 2011;31:6915-6927.

Fowden AL. The insulin-like growth factors and feto-placental growth. *Placenta* 2003;24:803-812.

Franco B, Guioli S, Pragliola A, Incerti B, Bardoni B, Tonlorenzi R, Carrozzo R, Maestrini E, Pieretti M, Taillon-Miller P, Brown CJ, Willard HF, Lawrence C, Graziella Persico M, Camerino G, Ballabio A. A gene deleted in Kallmann's syndrome shares homology with neural cell adhesion and axonal path-finding molecules. *Nature* 1991;353:529-536.

Friederici AD, Pannekamp A, Partsch CJ, Ulmen U, Oehler K, Schmutzler R, Hesse V. Sex hormone testosterone affects language organization in the infant brain. *Neuroreport* 2008;19:283-286.

Frisch RE. The right weight: body fat, menarche and fertility. *Proc Nutr Soc* 1994;53:113-129.

Fuqua JS. Treatment and outcomes of precocious puberty: an update. *J Clin Endocrinol Metab* 2013;98:2198-2207.

Garcia CJ, Espinoza A, Dinamarca V, Navarro O, Daneman A, Garcia H, Cattani A. Breast US in children and adolescents. *Radiographics* 2000;20:1605-1612.

Garcia-Gonzalez D, Murcia-Belmonte V, Esteban PF, Ortega F, Diaz D, Sanchez-Vera I, Lebron-Galan R, Escobar-Castanondo L, Martinez-Millan L, Weruaga E, Garcia-Verdugo JM, Berninger B, de Castro F. Anosmin-1 over-expression increases adult neurogenesis in the subventricular zone and neuroblast migration to the olfactory bulb. *Brain Struct Funct* 2016;221:239-260.

Garnett SP, Hogler W, Blades B, Baur LA, Peat J, Lee J, Cowell CT. Relation between hormones and body composition, including bone, in prepubertal children. *Am J Clin Nutr* 2004;80:966-972.

Gianetti E, Tusset C, Noel SD, Au MG, Dwyer AA, Hughes VA, Abreu AP, Carroll J, Trarbach E, Silveira LF, Costa EM, de Mendonca BB, de Castro M, Lofrano A, Hall JE, Bolu E, Ozata M, Quinton R, Amory JK, Stewart SE, Arlt W, Cole TR, Crowley WF, Kaiser UB, Latronico AC, Seminara SB. TAC3/TACR3 mutations reveal preferential activation of gonadotropin-releasing hormone release by neurokinin B in neonatal life followed by reversal in adulthood. *J Clin Endocrinol Metab* 2010;95:2857-2867.

Gicquel C, Rossignol S, Cabrol S, Houang M, Steunou V, Barbu V, Danton F, Thibaud N, Le Merrer M, Burglen L, Bertrand AM, Netchine I, Le Bouc Y. Epimutation of the telomeric imprinting center region on chromosome 11p15 in Silver-Russell syndrome. *Nat Genet* 2005;37:1003-1007.

Goji K, Tanikaze S. Spontaneous gonadotropin and testosterone concentration profiles in prepubertal and pubertal boys: temporal relationship between luteinizing hormone and testosterone. *Pediatr Res* 1993;34:229-236.

Grachev P, Li XF, Lin YS, Hu MH, Elsamani L, Paterson SJ, Millar RP, Lightman SL, O'Byrne KT. GPR54-dependent stimulation of luteinizing hormone secretion by neurokinin B in prepubertal rats. *PLoS One* 2012;7:e44344.

Greulich, W.W. & Pyle, S.I. (eds) 1959, *Radiographic Atlas of Skeletal Development of the Hand and Wrist*, 2nd edn, Stanford University Press, Stanford, California, USA.

Grinspon RP, Ropelato MG, Gottlieb S, Keselman A, Martinez A, Ballerini MG, Domene HM, Rey RA. Basal follicle-stimulating hormone and peak gonadotropin levels after gonadotropin-releasing hormone infusion show high diagnostic accuracy in boys with suspicion of hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 2010;95:2811-2818.

Gross RT, Duke PM. The effect of early versus late physical maturation on adolescent behavior. *Pediatr Clin North Am* 1980;27:71-77.

Grumbach MM. A window of opportunity: the diagnosis of gonadotropin deficiency in the male infant. *J Clin Endocrinol Metab* 2005;90:3122-3127.

Gurbuz F, Kotan LD, Mengen E, Siklar Z, Berberoglu M, Dokmetas S, Kilicli MF, Guven A, Kirel B, Saka N, Poyrazoglu S, Cesur Y, Dogan M, Ozen S, Ozbek MN, Demirbilek H, Kekil MB, Temiz F, Onenli Mungan N, Yuksel B, Topaloglu AK. Distribution of gene mutations associated with familial normosmic idiopathic hypogonadotropic hypogonadism. *J Clin Res Pediatr Endocrinol* 2012;4:121-126.

Guthrie RD, Smith DW, Graham CB. Testosterone treatment for micropenis during early childhood. *J Pediatr* 1973;83:247-252.

Hagen CP, Sorensen K, Mieritz MG, Johannsen TH, Almstrup K, Juul A. Circulating MKRN3 Levels Decline Prior to Pubertal Onset and Through Puberty: A Longitudinal Study of Healthy Girls. *J Clin Endocrinol Metab* 2015;100:1920-1926.

Harrington J, Palmert MR. Clinical review: Distinguishing constitutional delay of growth and puberty from isolated hypogonadotropic hypogonadism: critical appraisal of available diagnostic tests. *J Clin Endocrinol Metab* 2012;97:3056-3067.

Hatipoglu N, Kurtoglu S. Micropenis: etiology, diagnosis and treatment approaches. *J Clin Res Pediatr Endocrinol* 2013;5:217-223.

Hayes FJ, Seminara SB, Decruz S, Boepple PA, Crowley WF,Jr. Aromatase inhibition in the human male reveals a hypothalamic site of estrogen feedback. *J Clin Endocrinol Metab* 2000;85:3027-3035.

Hellmann P, Christiansen P, Johannsen TH, Main KM, Duno M, Juul A. Male patients with partial androgen insensitivity syndrome: a longitudinal follow-up of growth, reproductive hormones and the development of gynaecomastia. *Arch Dis Child* 2012;97:403-409.

Herbison AE. Control of puberty onset and fertility by gonadotropin-releasing hormone neurons. *Nat Rev Endocrinol* 2016;12:452-466.

Hero M. Aromatase Inhibitors in the Treatment of Short Stature. *Endocr Dev* 2016;30:130-140.

Hero M, Ankarberg-Lindgren C, Taskinen MR, Dunkel L. Blockade of oestrogen biosynthesis in peripubertal boys: effects on lipid metabolism, insulin sensitivity, and body composition. *Eur J Endocrinol* 2006;155:453-460.

Hero M, Norjavaara E, Dunkel L. Inhibition of estrogen biosynthesis with a potent aromatase inhibitor increases predicted adult height in boys with idiopathic short stature: a randomized controlled trial. *J Clin Endocrinol Metab* 2005;90:6396-6402.

Hero M, Toiviainen-Salo S, Wickman S, Makitie O, Dunkel L. Vertebral morphology in aromatase inhibitor-treated males with idiopathic short stature or constitutional delay of puberty. *J Bone Miner Res* 2010;25:1536-1543.

Hero M, Tommiska J, Vaaralahti K, Laitinen EM, Sipila I, Puhakka L, Dunkel L, Raivio T. Circulating antimullerian hormone levels in boys decline during early puberty and correlate with inhibin B. *Fertil Steril* 2012;97:1242-1247.

Howard SR, Guasti L, Ruiz-Babot G, Mancini A, David A, Storr HL, Metherell LA, Sternberg MJ, Cabrera CP, Warren HR, Barnes MR, Quinton R, de Roux N, Young J, Guiochon-Mantel A, Wehkalampi K, Andre V, Gothilf Y, Cariboni A, Dunkel L. IGSF10 mutations dysregulate gonadotropin-releasing hormone neuronal migration resulting in delayed puberty. *EMBO Mol Med* 2016;8:626-642.

Huhtaniemi I. A short evolutionary history of FSH-stimulated spermatogenesis. *Hormones (Athens)* 2015;14:468-478.

Hutchins BI, Kotan LD, Taylor-Burds C, Ozkan Y, Cheng PJ, Gurbuz F, Tiong JD, Mengen E, Yuksel B, Topaloglu AK, Wray S. CCDC141 Mutation Identified in Anosmic Hypogonadotropic Hypogonadism (Kallmann Syndrome) Alters GnRH Neuronal Migration. *Endocrinology* 2016;157:1956-1966.

Ibanez L, Valls C, Cols M, Ferrer A, Marcos MV, De Zegher F. Hypersecretion of FSH in infant boys and girls born small for gestational age. *J Clin Endocrinol Metab* 2002;87:1986-1988.

Janfaza M, Sherman TI, Larmore KA, Brown-Dawson J, Klein KO. Estradiol levels and secretory dynamics in normal girls and boys as determined by an ultrasensitive bioassay: a 10 year experience. *J Pediatr Endocrinol Metab* 2006;19:901-909.

Janner M, Fluck CE, Mullis PE. Impact of estrogen replacement throughout childhood on growth, pituitary-gonadal axis and bone in a 46,XX patient with CYP19A1 deficiency. *Horm Res Paediatr* 2012;78:261-268.

Jiao H, Arner P, Dickson SL, Vidal H, Mejhert N, Henegar C, Taube M, Hansson C, Hinney A, Galan P, Simon C, Silveira A, Benrick A, Jansson JO, Bouloumie A, Langin D, Laville M, Debard C, Axelsson T, Ryden M, Kere J, Dahlman-Wright K, Hamsten A, Clement K, Dahlman I. Genetic association and gene expression analysis identify FGFR1 as a new susceptibility gene for human obesity. *J Clin Endocrinol Metab* 2011;96:E962-6.

Jin JM, Yang WX. Molecular regulation of hypothalamus-pituitary-gonads axis in males. *Gene* 2014;551:15-25.

Johnson W, Stovitz SD, Choh AC, Czerwinski SA, Towne B, Demerath EW. Patterns of linear growth and skeletal maturation from birth to 18 years of age in overweight young adults. *Int J Obes (Lond)* 2012;36:535-541.

Jong MT, Gray TA, Ji Y, Glenn CC, Saitoh S, Driscoll DJ, Nicholls RD. A novel imprinted gene, encoding a RING zinc-finger protein, and overlapping antisense transcript in the Prader-Willi syndrome critical region. *Hum Mol Genet* 1999;8:783-793.

Josso N, Picard JY, Tran D. Anti-mullerian hormone. *Reprod Nutr Dev* 1980;20:569-571.

Josso N, Rey RA, Picard JY. Anti-mullerian hormone: a valuable addition to the toolbox of the pediatric endocrinologist. *Int J Endocrinol* 2013;2013:674105.

Joustra SD, van der Plas EM, Goede J, Oostdijk W, Delemarre-van de Waal HA, Hack WW, van Buuren S, Wit JM. New reference charts for testicular volume in Dutch children and adolescents allow the calculation of standard deviation scores. *Acta Paediatr* 2015;104:e271-8.

Juul A. The effects of oestrogens on linear bone growth. *Hum Reprod Update* 2001;7:303-313.

Juul A, Scheike T, Nielsen CT, Krabbe S, Muller J, Skakkebaek NE. Serum insulin-like growth factor I (IGF-I) and IGF-binding protein 3 levels are increased in central precocious puberty: effects of two different treatment regimens with gonadotropin-releasing hormone agonists, without or in combination with an antiandrogen (cyproterone acetate). *J Clin Endocrinol Metab* 1995;80:3059-3067.

Juul A, Teilmann G, Scheike T, Hertel NT, Holm K, Laursen EM, Main KM, Skakkebaek NE. Pubertal development in Danish children: comparison of recent European and US data. *Int J Androl* 2006;29:247-55; discussion 286-90.

Kallmann FJ, Schonfeld WA, Barrera ES. THe Genetic Aspects of Primary Eunuchoidism. *American Journal of Mental Deficiency* 1944;48:203-236.

Kallo I, Vida B, Deli L, Molnar CS, Hrabovszky E, Caraty A, Ciofi P, Coen CW, Liposits Z. Co-localisation of kisspeptin with galanin or neurokinin B in afferents to mouse GnRH neurones. *J Neuroendocrinol* 2012;24:464-476.

Kalra B, Kumar A, Patel K, Patel A, Khosravi MJ. Development of a second generation Inhibin B ELISA. *J Immunol Methods* 2010;362:22-31.

Kaplan SL, Grumbach MM. The ontogenesis of human foetal hormones. II. Luteinizing hormone (LH) and follicle stimulating hormone (FSH). *Acta Endocrinol (Copenh)* 1976;81:808-829.

Kaplowitz P. Delayed puberty in obese boys: comparison with constitutional delayed puberty and response to testosterone therapy. *J Pediatr* 1998;133:745-749.

Karlberg J. A biologically-oriented mathematical model (ICP) for human growth. *Acta Paediatr Scand Suppl* 1989;350:70-94.

Katoh H, Shibata S, Fukuda K, Sato M, Satoh E, Nagoshi N, Minematsu T, Matsuzaki Y, Akazawa C, Toyama Y, Nakamura M, Okano H. The dual origin of the peripheral olfactory system: placode and neural crest. *Mol Brain* 2011;4:34-6606-4-34.

Kaushanski A, Laron Z. Growth pattern of boys with isolated gonadotropin deficiency. *Isr J Med Sci* 1979;15:518-521.

Kelly A, Winer KK, Kalkwarf H, Oberfield SE, Lappe J, Gilsanz V, Zemel BS. Age-based reference ranges for annual height velocity in US children. *J Clin Endocrinol Metab* 2014;99:2104-2112.

Kenealy BP, Kapoor A, Guerriero KA, Keen KL, Garcia JP, Kurian JR, Ziegler TE, Terasawa E. Neuroestradiol in the hypothalamus contributes to the regulation of gonadotropin releasing hormone release. *J Neurosci* 2013;33:19051-19059.

Kenealy BP, Keen KL, Kapoor A, Terasawa E. Neuroestradiol in the Stalk Median Eminence of Female Rhesus Macaques Decreases in Association With Puberty Onset. *Endocrinology* 2016;157:70-76.

Kim KH, Patel L, Tobet SA, King JC, Rubin BS, Stopa EG. Gonadotropin-releasing hormone immunoreactivity in the adult and fetal human olfactory system. *Brain Res* 1999;826:220-229.

Kindblom JM, Gothe S, Forrest D, Tornell J, Tornell J, Vennstrom B, Ohlsson C. GH substitution reverses the growth phenotype but not the defective ossification in thyroid hormone receptor alpha 1-/-beta-/- mice. *J Endocrinol* 2001;171:15-22.

Kiviranta P, Kuiri-Hanninen T, Saari A, Lamidi ML, Dunkel L, Sankilampi U. Transient Postnatal Gonadal Activation and Growth Velocity in Infancy. *Pediatrics* 2016;138:.2015-3561.

Krams M, Quinton R, Ashburner J, Friston KJ, Frackowiak RS, Bouloux PM, Passingham RE. Kallmann's syndrome: mirror movements associated with bilateral corticospinal tract hypertrophy. *Neurology* 1999;52:816-822.

Kuiri-Hanninen T, Haanpaa M, Turpeinen U, Hamalainen E, Seuri R, Tyrvainen E, Sankilampi U, Dunkel L. Postnatal ovarian activation has effects in estrogen target tissues in infant girls. *J Clin Endocrinol Metab* 2013;98:4709-4716.

Kuiri-Hanninen T, Kallio S, Seuri R, Tyrvainen E, Liakka A, Tapanainen J, Sankilampi U, Dunkel L. Postnatal developmental changes in the pituitary-ovarian axis in preterm and term infant girls. *J Clin Endocrinol Metab* 2011;96:3432-3439.

Kuiri-Hanninen T, Sankilampi U, Dunkel L. Activation of the hypothalamic-pituitary-gonadal axis in infancy: minipuberty. *Horm Res Paediatr* 2014;82:73-80.

Kuiri-Hanninen T, Seuri R, Tyrvainen E, Turpeinen U, Hamalainen E, Stenman UH, Dunkel L, Sankilampi U. Increased activity of the hypothalamic-pituitary-testicular axis in infancy results in increased androgen action in premature boys. *J Clin Endocrinol Metab* 2011;96:98-105.

Kusec V, Virdi AS, Prince R, Triffitt JT. Localization of estrogen receptor-alpha in human and rabbit skeletal tissues. *J Clin Endocrinol Metab* 1998;83:2421-2428.

Laitinen, E.M. Kallmann syndrome: clinical and molecular genetic features in Finland, 2012, University of Helsinki.

Laitinen EM, Hero M, Vaaralahti K, Tommiska J, Raivio T. Bone mineral density, body composition and bone turnover in patients with congenital hypogonadotropic hypogonadism. *Int J Androl* 2012a;35:534-540.

Laitinen EM, Tommiska J, Sane T, Vaaralahti K, Toppari J, Raivio T. Reversible congenital hypogonadotropic hypogonadism in patients with CHD7, FGFR1 or GNRHR mutations. *PLoS One* 2012b;7:e39450.

Laitinen EM, Vaaralahti K, Tommiska J, Eklund E, Tervaniemi M, Valanne L, Raivio T. Incidence, phenotypic features and molecular genetics of Kallmann syndrome in Finland. *Orphanet J Rare Dis* 2011;6:41-1172-6-41.

Lamminmaki A, Hines M, Kuiri-Hanninen T, Kilpelainen L, Dunkel L, Sankilampi U. Testosterone measured in infancy predicts subsequent sex-typed behavior in boys and in girls. *Horm Behav* 2012;61:611-616.

Landier F, Chaussain JL, Job JC. Early treatment of congenital hypoplasia of the penis with intramuscular delayed-action testosterone. *Arch Fr Pediatr* 1984;41:467-471.

Lasaite L, Ceponis J, Preiksa RT, Zilaitiene B. Impaired emotional state, quality of life and cognitive functions in young hypogonadal men. *Andrologia* 2013;6:1107-12.

Lass N, Kleber M, Winkel K, Wunsch R, Reinehr T. Effect of lifestyle intervention on features of polycystic ovarian syndrome, metabolic syndrome, and intima-media thickness in obese adolescent girls. *J Clin Endocrinol Metab* 2011;96:3533-3540.

Lawaetz JG, Hagen CP, Mieritz MG, Blomberg Jensen M, Petersen JH, Juul A. Evaluation of 451 Danish boys with delayed puberty: diagnostic use of a new puberty nomogram and effects of oral testosterone therapy. *J Clin Endocrinol Metab* 2015;100:1376-1385.

Lee JM, Wasserman R, Kaciroti N, Gebremariam A, Steffes J, Dowshen S, Harris D, Serwint J, Abney D, Smitherman L, Reiter E, Herman-Giddens ME. Timing of Puberty in Overweight Versus Obese Boys. *Pediatrics* 2016;137:e20150164.

Lee PA. Normal ages of pubertal events among American males and females. *J Adolesc Health Care* 1980;1:26-29.

Lee PA, O'Leary LA, Songer NJ, Coughlin MT, Bellinger MF, LaPorte RE. Paternity after unilateral cryptorchidism: a controlled study. *Pediatrics* 1996;98:676-679.

Legouis R, Hardelin JP, Levilliers J, Claverie JM, Compain S, Wunderle V, Millasseau P, Le Paslier D, Cohen D, Caterina D. The candidate gene for the X-linked Kallmann syndrome encodes a protein related to adhesion molecules. *Cell* 1991;67:423-435.

Lei ZM, Rao CV. Novel presence of luteinizing hormone/human chorionic gonadotropin (hCG) receptors and the down-regulating action of hCG on gonadotropin-releasing hormone gene expression in immortalized hypothalamic GT1-7 neurons. *Mol Endocrinol* 1994;8:1111-1121.

Lewkowitz-Shpuntoff HM, Hughes VA, Plummer L, Au MG, Doty RL, Seminara SB, Chan YM, Pitteloud N, Crowley WF,Jr, Balasubramanian R. Olfactory phenotypic spectrum in idiopathic hypogonadotropic hypogonadism: pathophysiological and genetic implications. *J Clin Endocrinol Metab* 2012;97:E136-44.

Liu JL, Yakar S, LeRoith D. Conditional knockout of mouse insulin-like growth factor-1 gene using the Cre/loxP system. *Proc Soc Exp Biol Med* 2000;223:344-351.

Lomniczi A, Wright H, Castellano JM, Matagne V, Toro CA, Ramaswamy S, Plant TM, Ojeda SR. Epigenetic regulation of puberty via Zinc finger protein-mediated transcriptional repression. *Nat Commun* 2015;6:10195.

Low LC, Tam SY, Kwan EY, Tsang AM, Karlberg J. Onset of significant GH dependence of serum IGF-I and IGF-binding protein 3 concentrations in early life. *Pediatr Res* 2001;50:737-742.

Lui JC, Nilsson O, Chan Y, Palmer CD, Andrade AC, Hirschhorn JN, Baron J. Synthesizing genome-wide association studies and expression microarray reveals novel genes that act in the human growth plate to modulate height. *Hum Mol Genet* 2012;21:5193-5201.

MacArthur DG, Manolio TA, Dimmock DP, Rehm HL, Shendure J, Abecasis GR, Adams DR, Altman RB, Antonarakis SE, Ashley EA, Barrett JC, Biesecker LG, Conrad DF, Cooper GM, Cox NJ, Daly MJ, Gerstein MB, Goldstein DB, Hirschhorn JN, Leal SM, Pennacchio LA, Stamatoyannopoulos JA, Sunyaev SR, Valle D, Voight BF, Winckler W, Gunter C. Guidelines for investigating causality of sequence variants in human disease. *Nature* 2014;508:469-476.

Macedo DB, Abreu AP, Reis AC, Montenegro LR, Dauber A, Beneduzzi D, Cukier P, Silveira LF, Teles MG, Carroll RS, Junior GG, Filho GG, Gucev Z, Arnhold IJ, de Castro M, Moreira AC, Martinelli CE,Jr, Hirschhorn JN, Mendonca BB, Brito VN, Antonini SR, Kaiser UB, Latronico AC. Central precocious puberty that appears to be sporadic caused by paternally inherited mutations in the imprinted gene makorin ring finger 3. *J Clin Endocrinol Metab* 2014;99:E1097-103.

Main KM, Schmidt IM, Skakkebaek NE. A possible role for reproductive hormones in newborn boys: progressive hypogonadism without the postnatal testosterone peak. *J Clin Endocrinol Metab* 2000;85:4905-4907.

Marshall WA. Interrelationships of skeletal maturation, sexual development and somatic growth in man. *Ann Hum Biol* 1974;1:29-40.

Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. *Arch Dis Child* 1970;45:13-23.

Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. *Arch Dis Child* 1969;44:291-303.

Mason AJ, Hayflick JS, Zoeller RT, Young WS,3rd, Phillips HS, Nikolics K, Seeburg PH. A deletion truncating the gonadotropin-releasing hormone gene is responsible for hypogonadism in the hpg mouse. *Science* 1986;234:1366-1371.

Matthews JN, Altman DG, Campbell MJ, Royston P. Analysis of serial measurements in medical research. *BMJ* 1990;300:230-235.

Mauras N, Blizzard RM, Link K, Johnson ML, Rogol AD, Veldhuis JD. Augmentation of growth hormone secretion during puberty: evidence for a pulse amplitude-modulated phenomenon. *J Clin Endocrinol Metab* 1987;64:596-601.

Mauras N, Gonzalez de Pijem L, Hsiang HY, Desrosiers P, Rapaport R, Schwartz ID, Klein KO, Singh RJ, Miyamoto A, Bishop K. Anastrozole increases predicted adult height of short adolescent males treated with growth hormone: a randomized, placebo-controlled, multicenter trial for one to three years. *J Clin Endocrinol Metab* 2008;93:823-831.

Mauras N, O'Brien KO, Klein KO, Hayes V. Estrogen suppression in males: metabolic effects. *J Clin Endocrinol Metab* 2000;85:2370-2377.

Mauras N, Rini A, Welch S, Sager B, Murphy SP. Synergistic effects of testosterone and growth hormone on protein metabolism and body composition in prepubertal boys. *Metabolism* 2003;52:964-969.

Merke DP, Keil MF, Jones JV, Fields J, Hill S, Cutler BG Jr. Flutamide, testolactone, and reduced hydrocortisone dose maintain normal growth velocity and bone maturation dispite elevated androgen levels in children with congenital adrenal hyperplasia. *J Clin Endocrinol Metab* 2000;85:1114-1120.

Miles HL, Gidlof S, Nordenstrom A, Ong KK, Hughes IA. The role of androgens in fetal growth: observational study in two genetic models of disordered androgen signalling. *Arch Dis Child Fetal Neonatal Ed* 2010;95:F435-8.

Millar RP, Newton CL. Current and future applications of GnRH, kisspeptin and neurokinin B analogues. *Nat Rev Endocrinol* 2013;9:451-466.

Mitamura R, Yano K, Suzuki N, Ito Y, Makita Y, Okuno A. Diurnal rhythms of luteinizing hormone, follicle-stimulating hormone, testosterone, and estradiol secretion before the onset of female puberty in short children. *J Clin Endocrinol Metab* 2000;85:1074-1080.

Mitamura R, Yano K, Suzuki N, Ito Y, Makita Y, Okuno A. Diurnal rhythms of luteinizing hormone, follicle-stimulating hormone, and testosterone secretion before the onset of male puberty. *J Clin Endocrinol Metab* 1999;84:29-37.

Molinari L, Gasser T, Largo R. A comparison of skeletal maturity and growth. *Ann Hum Biol* 2013;40:333-340.

Molsted K, Kjaer I, Giwercman A, Vesterhauge S, Skakkebaek NE. Craniofacial morphology in patients with Kallmann's syndrome with and without cleft lip and palate. *Cleft Palate Craniofac J* 1997;34:417-424.

Morishima A, Grumbach MM, Simpson ER, Fisher C, Qin K. Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens. *J Clin Endocrinol Metab* 1995;80:3689-3698.

Mul D, Fredriks AM, van Buuren S, Oostdijk W, Verloove-Vanhorick SP, Wit JM. Pubertal development in The Netherlands 1965-1997. *Pediatr Res* 2001;50:479-486.

Muller J, Juul A, Andersson AM, Sehested A, Skakkebaek NE. Hormonal changes during GnRH analogue therapy in children with central precocious puberty. *J Pediatr Endocrinol Metab* 2000;13 Suppl 1:739-746.

Muller J, Skakkebaek NE. Fluctuations in the number of germ cells during the late foetal and early postnatal periods in boys. *Acta Endocrinol (Copenh)* 1984;105:271-274.

Murray PG, Clayton PE. Endocrine control of growth. *Am J Med Genet C Semin Med Genet* 2013;163C:76-85.

Narayanaswamy S, Prague JK, Jayasena CN, Papadopoulou DA, Mizamtsidi M, Shah AJ, Bassett P, Comninos AN, Abbara A, Bloom SR, Veldhuis JD, Dhillo WS. Investigating the KNDy hypothesis in humans by co-administration of kisspeptin, neurokinin B and naltrexone in men. *J Clin Endocrinol Metab* 2016;jc20161911.

Navarro VM, Gottsch ML, Chavkin C, Okamura H, Clifton DK, Steiner RA. Regulation of gonadotropin-releasing hormone secretion by kisspeptin/dynorphin/neurokinin B neurons in the arcuate nucleus of the mouse. *J Neurosci* 2009;29:11859-11866.

Neely EK, Kumar RB, Payne SL, Ranadive SA, Suchet DI. Letrozole vs anastrozole for height augmentation in short pubertal males: first year data. *J Clin Endocrinol Metab* 2014;99:4086-4093.

Netchine I, Azzi S, Le Bouc Y, Savage MO. IGF1 molecular anomalies demonstrate its critical role in fetal, postnatal growth and brain development. *Best Pract Res Clin Endocrinol Metab* 2011;25:181-190.

Oerter KE, Uriarte MM, Rose SR, Barnes KM, Cutler GB,Jr. Gonadotropin secretory dynamics during puberty in normal girls and boys. *J Clin Endocrinol Metab* 1990;71:1251-1258.

Ojajärvi, P. The adolescent Finnish child: a longitudinal study of anthropometry, physical development, and physiological changes during puberty, 1982, University of Helsinki.

Ong KK, Langkamp M, Ranke MB, Whitehead K, Hughes IA, Acerini CL, Dunger DB. Insulin-like growth factor I concentrations in infancy predict differential gains in body length and adiposity: the Cambridge Baby Growth Study. *Am J Clin Nutr* 2009;90:156-161.

Ong KK, Potau N, Petry CJ, Jones R, Ness AR, Honour JW, de Zegher F, Ibanez L, Dunger DB, Avon Longitudinal Study of Parents and Children Study Team. Opposing influences of prenatal and postnatal weight gain on adrenarche in normal boys and girls. *J Clin Endocrinol Metab* 2004;89:2647-2651.

O'Shaughnessy PJ. Hormonal control of germ cell development and spermatogenesis. *Semin Cell Dev Biol* 2014;29:55-65.

Osuna JA, Arata-Bellabarba G, Tortolero MI. GnRH and hCG tests in healthy adolescents and adults. *Arch Androl* 2001;47:9-14.

Palmert MR, Dunkel L. Clinical practice. Delayed puberty. *N Engl J Med* 2012;366:443-453.

Pedersen JF. Fetal crown-rump length measurement by ultrasound in normal pregnancy. *Br J Obstet Gynaecol* 1982;89:926-930.

Piet R, de Croft S, Liu X, Herbison AE. Electrical properties of kisspeptin neurons and their regulation of GnRH neurons. *Front Neuroendocrinol* 2015;36:15-27.

Pingault V, Bodereau V, Baral V, Marcos S, Watanabe Y, Chaoui A, Fouveaut C, Leroy C, Verier-Mine O, Francannet C, Dupin-Deguine D, Archambeaud F, Kurtz FJ, Young J, Bertherat J, Marlin S, Goossens M, Hardelin JP, Dode C, Bondurand N. Loss-of-function mutations in SOX10 cause Kallmann syndrome with deafness. *Am J Hum Genet* 2013;92:707-724.

Pinilla L, Aguilar E, Dieguez C, Millar RP, Tena-Sempere M. Kisspeptins and reproduction: physiological roles and regulatory mechanisms. *Physiol Rev* 2012;92:1235-1316.

Pitteloud N, Acierno JS,Jr, Meysing A, Eliseenkova AV, Ma J, Ibrahimi OA, Metzger DL, Hayes FJ, Dwyer AA, Hughes VA, Yialamas M, Hall JE, Grant E, Mohammadi M, Crowley WF,Jr. Mutations in fibroblast growth factor receptor 1 cause both Kallmann syndrome and normosmic idiopathic hypogonadotropic hypogonadism. *Proc Natl Acad Sci U S A* 2006;103:6281-6286.

Pitteloud N, Durrani S, Raivio T, Sykiotis GP. Complex genetics in idiopathic hypogonadotropic hypogonadism. *Front Horm Res* 2010;39:142-153.

Polari L, Yatkin E, Martinez Chacon MG, Ahotupa M, Smeds A, Strauss L, Zhang F, Poutanen M, Saarinen N, Makela SI. Weight gain and inflammation regulate aromatase expression in male adipose tissue, as evidenced by reporter gene activity. *Mol Cell Endocrinol* 2015;412:123-130.

Prader A. Testicular size: assessment and clinical importance. *Triangle* 1966;7:240-243.

Quast A, Hesse V, Hain J, Wermke P, Wermke K. Baby babbling at five months linked to sex hormone levels in early infancy. *Infant Behav Dev* 2016;44:1-10.

Raboch J, Reisenauer R. Analysis of body height in 829 patients with different forms of testicular pathology. *Andrologia* 1976;8:265-268.

Raivio, T. 2013, Puberteettikehitys ja sen häiriöt in *Lääkärin käsikirja,* Duodecim.

Raivio T, Falardeau J, Dwyer A, Quinton R, Hayes FJ, Hughes VA, Cole LW, Pearce SH, Lee H, Boepple P, Crowley WF,Jr, Pitteloud N. Reversal of idiopathic hypogonadotropic hypogonadism. *N Engl J Med* 2007;357:863-873.

Raivio T, Sidis Y, Plummer L, Chen H, Ma J, Mukherjee A, Jacobson-Dickman E, Quinton R, Van Vliet G, Lavoie H, Hughes VA, Dwyer A, Hayes FJ, Xu S, Sparks S, Kaiser UB, Mohammadi M, Pitteloud N. Impaired fibroblast growth factor receptor 1 signaling as a cause of normosmic idiopathic hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 2009;94:4380-4390.

Raivio T, Wikstrom AM, Dunkel L. Treatment of gonadotropin-deficient boys with recombinant human FSH: long-term observation and outcome. *Eur J Endocrinol* 2007;156:105-111.

Reindollar RH, McDonough PG. Etiology and evaluation of delayed sexual development. *Pediatr Clin North Am* 1981;28:267-286.

Reyes FI, Winter JS, Faiman C. Studies on human sexual development. I. Fetal gonadal and adrenal sex steroids. *J Clin Endocrinol Metab* 1973;37:74-78.

Rikken B, Wit JM. Prepubertal height velocity references over a wide age range. *Arch Dis Child* 1992;67:1277-1280.

Rock KL, Gramm C, Rothstein L, Clark K, Stein R, Dick L, Hwang D, Goldberg AL. Inhibitors of the proteasome block the degradation of most cell proteins and the generation of peptides presented on MHC class I molecules. *Cell* 1994;78:761-771.

Roelants M, Hauspie R, Hoppenbrouwers K. References for growth and pubertal development from birth to 21 years in Flanders, Belgium. *Ann Hum Biol* 2009;36:680-694.

Rogol AD, Roemmich JN, Clark PA. Growth at puberty. *J Adolesc Health* 2002;31:192-200.

Rohayem J, Nieschlag E, Kliesch S, Zitzmann M. Inhibin B, AMH, but not INSL3, IGF1 or DHEAS support differentiation between constitutional delay of growth and puberty and hypogonadotropic hypogonadism. *Andrology* 2015;3:882-887.

Rosenfeld RG. Molecular mechanisms of IGF-I deficiency. *Horm Res* 2006;65 Suppl 1:15-20.

Rosenfield RL, Bordini B, Yu C. Comparison of detection of normal puberty in girls by a hormonal sleep test and a gonadotropin-releasing hormone agonist test. *J Clin Endocrinol Metab* 2013;98:1591-1601.

Rosenfield RL, Bordini B, Yu C. Comparison of detection of normal puberty in boys by a hormonal sleep test and a gonadotropin-releasing hormone agonist test. *J Clin Endocrinol Metab* 2012;97:4596-4604.

Saari A, Sankilampi U, Hannila ML, Kiviniemi V, Kesseli K, Dunkel L. New Finnish growth references for children and adolescents aged 0 to 20 years: Length/height-for-age, weight-for-length/height, and body mass index-for-age. *Ann Med* 2011;43:235-248.

Sabado V, Barraud P, Baker CV, Streit A. Specification of GnRH-1 neurons by antagonistic FGF and retinoic acid signaling. *Dev Biol* 2012;362:254-262.

Sadov S, Koskenniemi JJ, Virtanen HE, Perheentupa A, Petersen JH, Skakkebaek NE, Main KM, Toppari J. Testicular Growth During Puberty in Boys With and Without a History of Congenital Cryptorchidism. *J Clin Endocrinol Metab* 2016;101:2570-2577.

Salenave S, Chanson P, Bry H, Pugeat M, Cabrol S, Carel JC, Murat A, Lecomte P, Brailly S, Hardelin JP, Dode C, Young J. Kallmann's syndrome: a comparison of the reproductive phenotypes in men carrying KAL1 and FGFR1/KAL2 mutations. *J Clin Endocrinol Metab* 2008;93:758-763.

Sankilampi U, Saari A, Laine T, Miettinen PJ, Dunkel L. Use of electronic health records for automated screening of growth disorders in primary care. *JAMA* 2013;310:1071-1072.

Schally AV, Arimura A, Kastin AJ, Matsuo H, Baba Y, Redding TW, Nair RM, Debeljuk L, White WF. Gonadotropin-releasing hormone: one polypeptide regulates secretion of luteinizing and follicle-stimulating hormones. *Science* 1971;173:1036-1038.

Scherdel P, Dunkel L, van Dommelen P, Goulet O, Salaun JF, Brauner R, Heude B, Chalumeau M. Growth monitoring as an early detection tool: a systematic review. *Lancet Diabetes Endocrinol* 2016;4:447-456.

Schmid C, Zwimpfer C, Brandle M, Krayenbuhl PA, Zapf J, Wiesli P. Effect of thyroxine replacement on serum IGF-I, IGFBP-3 and the acid-labile subunit in patients with hypothyroidism and hypopituitarism. *Clin Endocrinol (Oxf)* 2006;65:706-711.

Schwabe JW, Klug A. Zinc mining for protein domains. *Nat Struct Biol* 1994;1:345-349.

Schwanzel-Fukuda M, Pfaff DW. Origin of luteinizing hormone-releasing hormone neurons. *Nature* 1989;338:161-164.

Sedlmeyer IL, Palmert MR. Delayed puberty: analysis of a large case series from an academic center. *J Clin Endocrinol Metab* 2002;87:1613-1620.

Segal TY, Mehta A, Anazodo A, Hindmarsh PC, Dattani MT. Role of gonadotropin-releasing hormone and human chorionic gonadotropin stimulation tests in differentiating patients with hypogonadotropic hypogonadism from those with constitutional delay of growth and puberty. *J Clin Endocrinol Metab* 2009;94:780-785.

Sehested A, Juul AA, Andersson AM, Petersen JH, Jensen TK, Muller J, Skakkebaek NE. Serum inhibin A and inhibin B in healthy prepubertal, pubertal, and adolescent girls and adult women: relation to age, stage of puberty, menstrual cycle, follicle-stimulating hormone, luteinizing hormone, and estradiol levels. *J Clin Endocrinol Metab* 2000;85:1634-1640.

Seminara SB, Messager S, Chatzidaki EE, Thresher RR, Acierno JS,Jr, Shagoury JK, Bo-Abbas Y, Kuohung W, Schwinof KM, Hendrick AG, Zahn D, Dixon J, Kaiser UB, Slaugenhaupt SA, Gusella JF, O'Rahilly S, Carlton MB, Crowley WF,Jr, Aparicio SA, Colledge WH. The GPR54 gene as a regulator of puberty. *N Engl J Med* 2003;349:1614-1627.

Sequera AM, Fideleff HL, Boquete HR, Pujol AB, Suarez MG, Ruibal GF. Basal ultrasensitive LH assay: a useful tool in the early diagnosis of male pubertal delay? *J Pediatr Endocrinol Metab* 2002;15:589-596.

Shaw ND, Seminara SB, Welt CK, Au MG, Plummer L, Hughes VA, Dwyer AA, Martin KA, Quinton R, Mericq V, Merino PM, Gusella JF, Crowley WF,Jr, Pitteloud N, Hall JE. Expanding the phenotype and genotype of female GnRH deficiency. *J Clin Endocrinol Metab* 2011;96:E566-76.

Shibata M, Friedman RL, Ramaswamy S, Plant TM. Evidence that down regulation of hypothalamic KiSS-1 expression is involved in the negative feedback action of testosterone to regulate luteinising hormone secretion in the adult male rhesus monkey (Macaca mulatta). *J Neuroendocrinol* 2007;19:432-438.

Shim KS. Pubertal growth and epiphyseal fusion. *Ann Pediatr Endocrinol Metab* 2015;20:8-12.

Shiraishi K, Oka S, Matsuyama H. Assessment of quality of life during gonadotrophin treatment for male hypogonadotrophic hypogonadism. *Clin Endocrinol (Oxf)* 2014;81:259-65.

Sidhoum VF, Chan YM, Lippincott MF, Balasubramanian R, Quinton R, Plummer L, Dwyer A, Pitteloud N, Hayes FJ, Hall JE, Martin KA, Boepple PA, Seminara SB. Reversal and relapse of hypogonadotropic hypogonadism: resilience and fragility of the reproductive neuroendocrine system. *J Clin Endocrinol Metab* 2014;99:861-870.

Silveira LG, Noel SD, Silveira-Neto AP, Abreu AP, Brito VN, Santos MG, Bianco SD, Kuohung W, Xu S, Gryngarten M, Escobar ME, Arnhold IJ, Mendonca BB, Kaiser UB, Latronico AC. Mutations of the KISS1 gene in disorders of puberty. *J Clin Endocrinol Metab* 2010;95:2276-2280.

Simon D, Ba I, Mekhail N, Ecosse E, Paulsen A, Zenaty D, Houang M, Jesuran-Perelroizan M, De Filippo G, Salerno M, Simonin G, Reynaud R, Carel JC, Leger J, De Roux N. Mutations in the Maternally Imprinted Gene MKRN3 Are Common in Familial Central Precocious Puberty. *Eur J Endocrinol* 2016;174(1):1-8.

Simonneaux V, Bahougne T. A Multi-Oscillatory Circadian System Times Female Reproduction. *Front Endocrinol (Lausanne)* 2015;6:157.

Simorangkir DR, Marshall GR, Plant TM. Sertoli cell proliferation during prepubertal development in the rhesus monkey (Macaca mulatta) is maximal during infancy when gonadotropin secretion is robust. *J Clin Endocrinol Metab* 2003;88:4984-4989.

Sintonen H. The 15D instrument of health-related quality of life: properties and applications. *Ann Med* 2001;33:328-336.

Skrapits K, Borsay BA, Herczeg L, Ciofi P, Liposits Z, Hrabovszky E. Neuropeptide co-expression in hypothalamic kisspeptin neurons of laboratory animals and the human. *Front Neurosci* 2015;9:29.

Smink JJ, Koster JG, Gresnigt MG, Rooman R, Koedam JA, Van Buul-Offers SC. IGF and IGF-binding protein expression in the growth plate of normal, dexamethasone-treated and human IGF-II transgenic mice. *J Endocrinol* 2002;175:143-153.

Socha P, Grote V, Gruszfeld D, Janas R, Demmelmair H, Closa-Monasterolo R, Subias JE, Scaglioni S, Verduci E, Dain E, Langhendries JP, Perrin E, Koletzko B, European Childhood Obesity Trial Study Group. Milk protein intake, the metabolic-endocrine response, and growth in infancy: data from a randomized clinical trial. *Am J Clin Nutr* 2011;94:1776S-1784S.

Soliman AT, Khadir MM, Asfour M. Testosterone treatment in adolescent boys with constitutional delay of growth and development. *Metabolism* 1995;44:1013-1015.

Sorensen K, Mouritsen A, Aksglaede L, Hagen CP, Mogensen SS, Juul A. Recent secular trends in pubertal timing: implications for evaluation and diagnosis of precocious puberty. *Horm Res Paediatr* 2012;77:137-145.

Sorva R, Lankinen S, Tolppanen EM, Perheentupa J. Variation of growth in height and weight of children. II. After infancy. *Acta Paediatr Scand* 1990;79:498-506.

Sorva R, Tolppanen EM, Perheentupa J. Variation of growth in length and weight of children. I. Years 1 and 2. *Acta Paediatr Scand* 1990;79:490-497.

Sparago A, Cerrato F, Vernucci M, Ferrero GB, Silengo MC, Riccio A. Microdeletions in the human H19 DMR result in loss of IGF2 imprinting and Beckwith-Wiedemann syndrome. *Nat Genet* 2004;36:958-960.

Stamou MI, Cox KH, Crowley WF,Jr. Discovering Genes Essential to the Hypothalamic Regulation of Human Reproduction Using a Human Disease Model: Adjusting to Life in the "-Omics" Era. *Endocr Rev* 2015;36:603-621.

Stevens DA, Hasserjian RP, Robson H, Siebler T, Shalet SM, Williams GR. Thyroid hormones regulate hypertrophic chondrocyte differentiation and expression of parathyroid hormone-related peptide and its receptor during endochondral bone formation. *J Bone Miner Res* 2000;15:2431-2442.

Stochholm K, Juul S, Juel K, Naeraa RW, Gravholt CH. Prevalence, incidence, diagnostic delay, and mortality in Turner syndrome. *J Clin Endocrinol Metab* 2006;91:3897-3902.

Sun SS, Schubert CM, Chumlea WC, Roche AF, Kulin HE, Lee PA, Himes JH, Ryan AS. National estimates of the timing of sexual maturation and racial differences among US children. *Pediatrics* 2002;110:911-919.

Tanner JM, Whitehouse RH, Cameron N, Marshall WA, Healy MJ & Goldstein H. 1983, Assessment of skeletal maturity and prediction of adult height*,* Academic Press, London.

Tanner JM, Whitehouse RH, Marubini E, Resele LF. The adolescent growth spurt of boys and girls of the Harpenden growth study. *Ann Hum Biol* 1976;3:109-126.

Tapanainen J, Koivisto M, Vihko R & Huhtaniemi I. Enhanced activity of the pituitary-gonadal axis in premature human infants. *J Clin Endocrinol Metab* 1981;52:235-238.

Taskinen S, Taavitsainen M, Wikstrom S. Measurement of testicular volume: comparison of 3 different methods. *J Urol* 1996;155:930-933.

Taylor M, Couto-Silva AC, Adan L, Trivin C, Sainte-Rose C, Zerah M, Valteau-Couanet D, Doz F, Chalumeau M, Brauner R. Hypothalamic-pituitary lesions in pediatric patients: endocrine symptoms often precede neuro-ophthalmic presenting symptoms. *J Pediatr* 2012;161:855-863.

Thiering E, Bruske I, Kratzsch J, Hoffmann B, Herbarth O, von Berg A, Schaaf B, Wichmann HE, Heinrich J, LISAplus Study Group. Peak growth velocity in infancy is positively associated with blood pressure in school-aged children. *J Hypertens* 2012;30:1114-1121.

Thodberg HH, Kreiborg S, Juul A, Pedersen KD. The BoneXpert method for automated determination of skeletal maturity. *IEEE Trans Med Imaging* 2009;28:52-66.

Tommiska J, Kansakoski J, Christiansen P, Jorgensen N, Lawaetz JG, Juul A, Raivio T. Genetics of congenital hypogonadotropic hypogonadism in Denmark. *Eur J Med Genet* 2014;57:345-348.

Tommiska J, Toppari J, Vaaralahti K, Kansakoski J, Laitinen EM, Noisa P, Kinnala A, Niinikoski H, Raivio T. PROKR2 mutations in autosomal recessive Kallmann syndrome. *Fertil Steril* 2013;99:815-818.

Topaloglu AK, Tello JA, Kotan LD, Ozbek MN, Yilmaz MB, Erdogan S, Gurbuz F, Temiz F, Millar RP, Yuksel B. Inactivating KISS1 mutation and hypogonadotropic hypogonadism. *N Engl J Med* 2012;366:629-635.

Toublanc JE, Roger M, Chaussain JL. Etiologies of late puberty. *Horm Res* 1991;36:136-140.

Tuchman S, Thayu M, Shults J, Zemel BS, Burnham JM, Leonard MB. Interpretation of biomarkers of bone metabolism in children: impact of growth velocity and body size in healthy children and chronic disease. *J Pediatr* 2008;153:484-490.

Uhlen M, Fagerberg L, Hallstrom BM, Lindskog C, Oksvold P, Mardinoglu A, Sivertsson A, Kampf C, Sjostedt E, Asplund A, Olsson I, Edlund K, Lundberg E, Navani S, Szigyarto CA, Odeberg J, Djureinovic D, Takanen JO, Hober S, Alm T, Edqvist PH, Berling H, Tegel H, Mulder J, Rockberg J, Nilsson P, Schwenk JM, Hamsten M, von Feilitzen K, Forsberg M, Persson L, Johansson F, Zwahlen M, von Heijne G, Nielsen J, Ponten F. Proteomics. Tissue-based map of the human proteome. *Science* 2015;347:1260419.

Underwood, L.E. & Van Wyk, J.J. 1992, Normal and aberrant growth Williams' textbook of Endocrinolgy in W.B Saunders, Philadelphia.

Uriarte MM, Baron J, Garcia HB, Barnes KM, Loriaux DL, Cutler GB,Jr. The effect of pubertal delay on adult height in men with isolated hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 1992;74:436-440.

Vaaralahti K, Tommiska J, Tillmann V, Liivak N, Kansakoski J, Laitinen EM, Raivio T. De novo SOX10 nonsense mutation in a patient with Kallmann syndrome and hearing loss. *Pediatr Res* 2014;76:115-116.

Vaaralahti K, Wehkalampi K, Tommiska J, Laitinen EM, Dunkel L, Raivio T. The role of gene defects underlying isolated hypogonadotropic hypogonadism in patients with constitutional delay of growth and puberty. *Fertil Steril* 2011;95:2756-2758.

Valdes-Socin H, Rubio Almanza M, Tome Fernandez-Ladreda M, Debray FG, Bours V, Beckers A. Reproduction, smell, and neurodevelopmental disorders: genetic defects in different hypogonadotropic hypogonadal syndromes. *Front Endocrinol (Lausanne)* 2014;5:109.

van Brakel J, Kranse R, de Muinck Keizer-Schrama SM, Hendriks AE, de Jong FH, Bangma CH, Hazebroek FW, Dohle GR. Fertility potential in men with a history of congenital undescended testes: a long-term follow-up study. *Andrology* 2013;1:100-108.

van Brakel J, Kranse R, de Muinck Keizer-Schrama SM, Hendriks AE, de Jong FH, Hack WW, van der Voort-Doedens LM, Bangma CH, Hazebroek FW, Dohle GR. Fertility potential in a cohort of 65 men with previously acquired undescended testes. *J Pediatr Surg* 2014;49:599-605.

Van Dop C, Burstein S, Conte FA, Grumbach MM. Isolated gonadotropin deficiency in boys: clinical characteristics and growth. *J Pediatr* 1987;111:684-692.

Varimo T, Hero M, Kansakoski J, Vaaralahti K, Matikainen N, Raivio T. Circulating makorin ring-finger protein-3 (MKRN3) levels in healthy men and in men with hypogonadotropic hypogonadism. *Clin Endocrinol (Oxf)* 2016;84:638.

Vastagh C, Schwirtlich M, Kwakowsky A, Erdelyi F, Margolis FL, Yanagawa Y, Katarova Z, Szabo G. The spatiotemporal segregation of GAD forms defines distinct GABA signaling functions in the developing mouse olfactory system and provides novel insights into the origin and migration of GnRH neurons. *Dev Neurobiol* 2015;75:249-270.

Venken K, Schuit F, Van Lommel L, Tsukamoto K, Kopchick JJ, Coschigano K, Ohlsson C, Moverare S, Boonen S, Bouillon R, Vanderschueren D. Growth without growth hormone receptor: estradiol is a major growth hormone-independent regulator of hepatic IGF-I synthesis. *J Bone Miner Res* 2005;20:2138-2149.

Villanueva C, Jacobson-Dickman E, Xu C, Manouvrier S, Dwyer AA, Sykiotis GP, Beenken A, Liu Y, Tommiska J, Hu Y, Tiosano D, Gerard M, Leger J, Drouin-Garraud V, Lefebvre H, Polak M, Carel JC, Phan-Hug F, Hauschild M, Plummer L, Rey JP, Raivio T, Bouloux P, Sidis Y, Mohammadi M, de Roux N, Pitteloud N. Congenital hypogonadotropic hypogonadism with split hand/foot malformation: a clinical entity with a high frequency of FGFR1 mutations. *Genet Med* 2015;17:651-659.

Volkl TM, Simm D, Beier C, Dorr HG. Obesity among children and adolescents with classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Pediatrics* 2006;117:e98-105.

Ward DR, Dear FM, Ward IA, Anderson SI, Spergel DJ, Smith PA, Ebling FJ. Innervation of gonadotropin-releasing hormone neurons by peptidergic neurons conveying circadian or energy balance information in the mouse. *PLoS One* 2009;4:e5322.

Watanabe M, Fukuda A, Nabekura J. The role of GABA in the regulation of GnRH neurons. *Front Neurosci* 2014;8:387.

Wehkalampi K, Pakkila K, Laine T, Dunkel L. Adult height in girls with delayed pubertal growth. *Horm Res Paediatr* 2011;76:130-135.

Wehkalampi K, Vangonen K, Laine T, Dunkel L. Progressive reduction of relative height in childhood predicts adult stature below target height in boys with constitutional delay of growth and puberty. *Horm Res* 2007;68:99-104.

Wickman S, Dunkel L. Inhibition of P450 aromatase enhances gonadotropin secretion in early and midpubertal boys: evidence for a pituitary site of action of endogenous E. *J Clin Endocrinol Metab* 2001;86:4887-4894.

Wierman ME, Kiseljak-Vassiliades K, Tobet S. Gonadotropin-releasing hormone (GnRH) neuron migration: initiation, maintenance and cessation as critical steps to ensure normal reproductive function. *Front Neuroendocrinol* 2011;32:43-52.

Winter JS, Hughes IA, Reyes FI, Faiman C. Pituitary-gonadal relations in infancy: 2. Patterns of serum gonadal steroid concentrations in man from birth to two years of age. *J Clin Endocrinol Metab* 1976;42:679-686.

Wit JM, Hero M, Nunez SB. Aromatase inhibitors in pediatrics. *Nat Rev Endocrinol* 2011;8:135-147.

Wohlfa-Veje C, Mouritsen A, Hagen CP, Tinggaard J, Mieritz MG, Boas M, Petersen JH, Skakkebaek NE, Main KM. Pubertal Onset in Boys and Girls Is Influenced by Pubertal Timing of Both Parents. *J Clin Endocrinol Metab* 2016;101:2667-2674.

Wood AR, Esko T, Yang J, Vedantam S, Pers TH, Gustafsson S, Chu AY, Estrada K, Luan J, Kutalik Z, Amin N, Buchkovich ML, Croteau-Chonka DC, Day FR, Duan Y, Fall T, Fehrmann R, Ferreira T, Jackson AU, Karjalainen J, Lo KS, Locke AE, Magi R, Mihailov E, Porcu E, Randall JC, Scherag A, Vinkhuyzen AA, Westra HJ, Winkler TW, Workalemahu T, Zhao JH, Absher D, Albrecht E, Anderson D, Baron J, Beekman M, Demirkan A, Ehret GB, Feenstra B, Feitosa MF, Fischer K, Fraser RM, Goel A, Gong J, Justice AE, Kanoni S, Kleber ME, and collaborators. Defining the role of common variation in the genomic and biological architecture of adult human height. *Nat Genet* 2014;46:1173-1186.

Wray S. From nose to brain: development of gonadotrophin-releasing hormone-1 neurones. *J Neuroendocrinol* 2010;22:743-753.

Wray S, Grant P, Gainer H. Evidence that cells expressing luteinizing hormone-releasing hormone mRNA in the mouse are derived from progenitor cells in the olfactory placode. *Proc Natl Acad Sci U S A* 1989;86:8132-8136.

Wu FC, Brown DC, Butler GE, Stirling HF, Kelnar CJ. Early morning plasma testosterone is an accurate predictor of imminent pubertal development in prepubertal boys. *J Clin Endocrinol Metab* 1993;76:26-31.

Wu FC, Butler GE, Kelnar CJ, Huhtaniemi I, Veldhuis JD. Ontogeny of pulsatile gonadotropin releasing hormone secretion from midchildhood, through puberty, to adulthood in the human male: a study

using deconvolution analysis and an ultrasensitive immunofluorometric assay. *J Clin Endocrinol Metab* 1996;81:1798-1805.

Wu T, Mendola P, Buck GM. Ethnic differences in the presence of secondary sex characteristics and menarche among US girls: the Third National Health and Nutrition Examination Survey, 1988-1994. *Pediatrics* 2002;110:752-757.

Yin X, Wang X, Hu X, Chen Y, Zeng K, Zhang H. ERbeta induces the differentiation of cultured osteoblasts by both Wnt/beta-catenin signaling pathway and estrogen signaling pathways. *Exp Cell Res* 2015;335:107-114.

Young J. Approach to the male patient with congenital hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 2012;97:707-718.

Zacharin M, Sabin MA, Nair VV, Dabadghao P. Addition of recombinant follicle-stimulating hormone to human chorionic gonadotropin treatment in adolescents and young adults with hypogonadotropic hypogonadism promotes normal testicular growth and may promote early spermatogenesis. *Fertil Steril* 2012;98:836-842.

Zarotsky V, Huang MY, Carman W, Morgentaler A, Singhal PK, Coffin D, Jones TH. Systematic literature review of the risk factors, comorbidities, and consequences of hypogonadism in men. *Andrology* 2014;2:819-834.

Zenaty D, Bretones P, Lambe C, Guemas I, David M, Leger J, de Roux N. Paediatric phenotype of Kallmann syndrome due to mutations of fibroblast growth factor receptor 1 (FGFR1). *Mol Cell Endocrinol* 2006;254-255:78-83.

ISBN 978-951-51-2838-6 (paperback)
ISBN 978-951-51-2839-3 (PDF)
http://ethesis.helsinki.fi
Painosalama Oy
Helsinki 2016